EVALUATION OF QUADRICEPS MUSCLE ENDURANCE WITH FUNCTIONAL NEAR INFRARED SPECROSCOPY (fNIRS)

by

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ABSTRACT

EVALUATION OF QUADRICEPS MUSCLE ENDURANCE WITH FUNCTIONAL NEAR INFRARED SPECROSCOPY (fNIRS)

Muscular endurance evaluation methods involve use of exhaustive exercise protocols, biochemical investigation procedures and Surface Electromyography (sEMG) as well as, various optical imaging techniques including Functional Near-Infra Red Spectroscopy (fNIRS). In this project it is aimed to assess levels of tissue deoxygenation trends in the contracting muscle during squat exercise via fNIRS, and electrical behaviour of the muscle during exercise evaluated via sEMG.

In the study, twelve healthy male subjects, comprised of trained and less trained or sedantary individuals, exercised unloaded squat with the knee angle at 70˚ flexion from full extantion untill fatigue set in. Both fNIRS and sEMG measurements gathered from Vastus Lateralis (VL) of the quadriceps muscle. Deoxyhemoglobin (HB), Oxyhemoglobin (HBO2) and Oxygenation (OXY) parameters of fNIRS measurements and Root Mean Square (RMS), Mean Frequency (MNF) and Median Frequency (MDF) parameters of sEMG measurements assessed between subjects who were able to maintain exercise longer than five minutes and shorter than five minutes.

The results of the study showed that in the less exercising subjects HBO2 amplitude is %54 and OXY apmlitude is %58 small relative to exercising subjects which is concluded to be a result of training induced physiologic adaptations leading to altered oxygenation and oxygen extraction capability of the exercising muscle. However, sEMG parameters did not show a specific distinction between two groups yet, provided an objective sight about fatigue occurrance rather than information from participants.

Keywords: Functional near-infrared spectroscopy (fNIRS), surface electromyography (sEMG), muscular endurance, training, vastus lateralis.

ÖZET

İŞLEVSEL YAKIN KIZIL ÖTESİ SPEKTROSKOPİ (fNIRS) İLE KUADRİSEPS KASI DAYANIKLILIĞININ DEĞERLENDİRİLMESİ

Kas dayanıklılığını değerlendirme metodları, yorucu egzersiz protokolleri, biokimyasal araştırmalar ve Yüzeyel Elektromiyografi (yEMG) kadar, İşlevsel Yakın-Kızıl Ötesi Spektroskopi (iYKÖS) dahil çeşitli optik görüntüleme tekniklerinin kullanımını da kapsar. Bu çalışmada, kasılmakta olan kastaki doku oksijenlenme trendlerinin iYKÖS ile ve kasın elektriksel davranışın yEMG ile değerlendirilmesi amaçlanmıştır.

Çalışmada, egzersiz yapanlar ve daha az egzersiz yapan veya sedanterlerden oluşan on iki sağlıklı erkek deneğe, kasta yorgunluk oluşuncaya kadar diz tam ekstansiyondan 70˚ fleksiyon sağlayacak şekilde ağırlıksız skuat egzersizi yaptırıldı. fNIRS ve yEMG ölçümlerinin her ikisi de kuadrisepsin Vastus Lateralis (VL) kasından elde edildi. İYKÖS ölçümlerinden Deoksihemoglobin (HB), Oksihemoglobin (HBO2) ve Oksijenlenme (OXY) parametreleri ile, yEMG ölçümlerinden Kök-Ortalama-Kare (KOK), Ortalama Frekans (OMF) ve Ortanca Frekans (OCF) parametreleri değerlendirildi.

Çalışmanın sonuçları, az egzersiz yapanlarda, çok egzersiz yapanlara göre HBO2 genliğinin %54 ve OXY genliğinin %58 küçük olduğunu göstermiştir ki bunun egzersiz yapan kasta, idmana bağlı fizyolojik adaptasyonların neden olduğu artmış oksijenlenme ve oksijen çekme kabilyetinin bir sonucu olduğu kanaatine varılmıştır. Ne var ki, yEMG parametre eğimlerinde iki grup arasında özel bir fark görülmemiştir ancak, yEMG kas yorgunluğu oluşumunda deneklerin subjektif verilerinden ziyade objektif görüş sağlamıştır.

Anahtar Sözcükler: İşlevsel yakın-kızıl ötesi spektroskopi (iYKÖS), yüzeyel elektromiyografi (yEMG), kas dayanıklılığı, egzersiz, vastus lateralis.

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Ag-AgCl	Silver-silver chloride
Ca^{+2}	Calcium ion
cm	Centimeter
CO_2	Carbondioxide
H^+	Hydrogen ion
HB	Deoxyhemoglobin
HBO_2	Oxyhemoglobin
$HC0_3^-$	Bicarbonate
hz	Hertz
H-NMRS	Proton nuclear magnetic resonance spectroscopy
K^+	Potassium ion
km	Kilometer
^{31}P-NMRS	31P-nuclear magnetic resonance spectroscopy
Na^+	Sodium ion
^{23}Na-NMRS	23Na- nuclear magnetic resonance spectroscopy
nm	Nano-meter
O_2	Oxygen
$PC0_2$	Intracellular pressure of carbondioxide
PCr	Phosphocreatine
pH	Potential of hydrogen
Pi	Inorganic phosphate
SO_2	Oxygen saturation
t1/2	Half recovery time
tHb	Total hemoglobin
VO_2max	Maximal oxygen consumption
α	Significance level

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BOLD	Blood oxygenation level dependent
BV	Blood volume
CKC	Closed chain exercise
COPD	Cronic obstructive pulmonary disease
CtOx	Cytochrome oxidase
EMG	Electromyography
EPOC	Excess post-exercise oxygen consumption
FF	Fast contracting fast fatigable
FG	Fast glycolytic
fMRI	Functional Magnetic Resonance Imaging
fNIRS	Functional near-infrared spectroscopy
FOG	Fast-oxydative-glycloytic
FR	Fast contracting fatigue resistant
FT	Fast twitch
iEMG	Integrated electromyography
LBP	Low back pain
LED	Light emitting diyote
MBF	Muscle blood flow
MDF	Median frequency
MFCV	Muscle fiber conduction velocity
MNF	Mean frequency
MRI	Magnetic Resonance Imaging
MRS	Magnetic resonance spectroscopy
MVC	Maximum voluntary contraction
NIR_{CWS}	Continuous wave
NIR_{PMS}	Phase modulation
NIRS	Near-infrared spectroscopy
$NIRS_{SRS}$	Continuous wave spatially resolved

NIR_{TRS}	Pulsed
PVD	Peripheral vascular disease
RF	Rectus femoris
RM	Repetition maximum
RMS	Root mean square
S	Slow contracting
sEMG	Surface electromyography
SO	Slow oxidative
VIC	Voluntary isometric contraction
VL	Vastus lateralis

1. INTRODUCTION

1.1 Motivation and Objective

The use of optical methods to investigate muscle oxidative metabolism led to the development and increasingly widespread use of Near-infrared spectroscopy (NIRS), which offers a safe, non-invasive means of monitoring cerebral and muscle function without the use of radioisotopes or other contrast agents [1]. It is a relatively new technique which allows the simultaneous measurement of changes in intravascular and mitochondrial oxygenation.

During recent decades, there is a growing interest in examining the changes in skeletal muscle respiration during a variety of exercise conditions [e.g. 2]. Scientists have successfully used this noninvasive technique to investigate the diseases associated with impaired tissue oxygenation as well as human muscle performance, oxygen consumption and metabolic processes.

Efforts for evaluating muscle performance expose muscular endurance as another issue to explore. In addition, to the use of various exhaustive exercise protocols, Electromyography (EMG) for evaluation of fatigue, Magnetic Resonance Spectroscopy (MRS) for chemical information -inorganic phosphate (Pi), phosphocreatine (PCr) and pH levels- about tissues under investigation, gas exchange and cardiac output measures, Doppler ultrasound for blood flow measures, are being utilised. At that point, Functional Near-Infrared Spectroscopy (fNIRS) emerges as a valuable method to assess levels of tissue oxygenation/deoxygenation trends in evaluating oxygen supply, which is one of the critical determinants of muscular endurance, to the contracting muscle during the effort.

The goal of this study was to measure the changes in muscle deoxygenation trends during a sustained isometric quadriceps endurance test using fNIRS by comparing the measurements obtained from the subjects, who are regularly exercising and relatively less exercising or sedantary subjects. The relationship between muscle deoxygenation and

endurance times was investigated and showing the results indicating increased oxygen extraction capability of the regularly exercising subjects during isometric endurance exercise was aimed at using fNIRS.

Additionally, neuromuscular activation and predictions of performance decrements during extended fatiguing task was investigated by means of sEMG.

1.2 Outline of the Thesis

In writing this thesis I have attempted to provide a thorough background of the physiological adaptations induced by training and factors which play role in the delay of fatigue consequently alter muscular endurance.

Chapter 2 contains a description of the physiology of the human skeletal muscle in general and provides information about muscle contraction. Chapter 3 provides a general introduction to the endurance assessment methods.

Chapter 4 describes the use of NIRS device in the literature and gives information about fNIRS. Chapter 5 contains information about EMG and EMG parameters' physiologic interpretations.

Chapter 6 describes experimental procedures and methodology including detailed information about instrumentation. Chapter 7 mentions the results gathered from fNIRS and sEMG and their physiologic meaning. The results are discussed in further detail in Chapter 8 and concluded in Chapter 9. In Appedix schematics of NIRS and EMG measurements take place.

2. SKELETAL MUSCLE

The human body contains over four hundred skeletal museles, which constitute 40-50 per cent of the total body weight. The muscles provide strength and protection to the skeleton by distributing loads and absorbing shock and enable the bones to move at the joints [3].

Skeletal muscle performs three important functions (i) dynamic work; force generation for locomotion, the positioning of the body segments in space and breathing, (ii) static work; force generation for postural support and (iii) heat production [3, 4].

2.1 Structure of Skeletal Muscle

Skeletal muscle is composed of several tissue types and basic unit of skeletal muscle is a fiber. A single muscle fiber is a cyclindrical, elongated cell. Each fiber is surrounded by a thin layer of connective tissue called endomysium. Thousands of muscle fibers are wrapped by a thin layer of connective tissue called the perimysium to form a muscle bundle. Groups of muscle bundles that join into a tendon at each end form the muscle belly [3]. Figure 2.1 represents diagrammatic breakdown of a typical muscle and Figure 2.2 depicts cross section of a muscle fiber.

The cell membrane of skeletal muscle is called sarcollemma which controls what enters and leaves the cell and contains regulatory proteins that are influenced by hormones like epinephrine (adrenalin) and insulin. The blood concentration of these hormones influences conciderably the fuel utilization by the muscle cell. The contractile machinery of a muscle fiber is organized into structural units called sarcomeres [3].

Skeletal muscle fibers contain an extensive sarcoplasmic reticulum that wraps around each myofibril like a piece of lace. The sarcoplasmic reticulum consists of longitudinal tubules, which release Ca^{+2}, and the terminal cisternae, which concentrate and

sequester Ca^{+2}. A branching network of transverse tubules, also known as t-tubules, is closely associated with the terminal cisternae [3, 4]. One t-tubule with its flanking terminal cisternae is known as a triad. The membranes of t-tubules are a continuation of the muscle fiber membrane. This makes the lumen of the t-tubules continuous with the extracellular fluid.

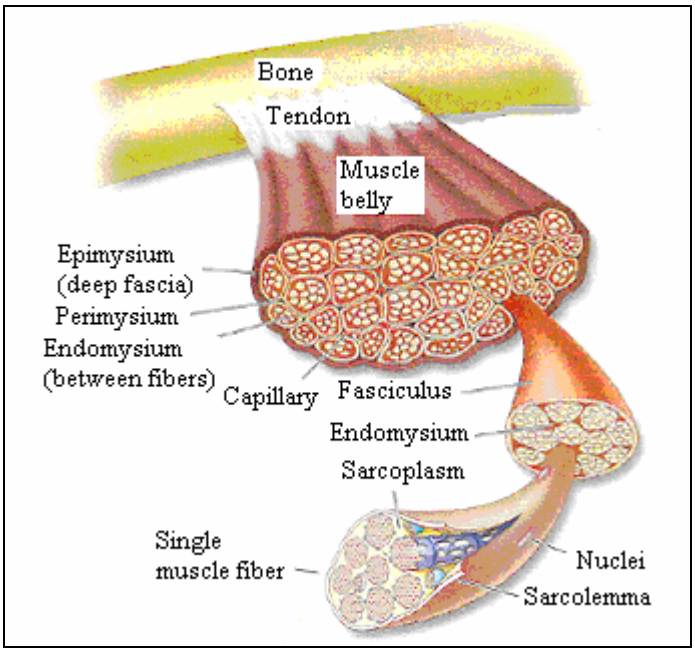

Figure 2.1 Diagrammatic breakdown of a typical muscle [4].

T-tubules allow action potentials that originate at the neuromuscular junction on the cell surface to move rapidly into the interior of the fiber. The cytosol between the myofibrils contains glycogen which is a reserve source of energy and mitochondria provide much of the adenosine triphosphate (ATP) for muscle contraction through oxidative phosphorylation of glucose and other biomolecules [4].

The organelles in each muscle cell that contain oxidative enzymes consume oxygen during exercise. Mitochondria convert the chemical energy contained in fat and carbo hydrate to ATP, the only energy source that can be used directly by the cell to support contraction. Ultimately, glucose and fat molecules (and certain amino acids) break down and combine with oxygen to form ATP, carbon dioxide, water, and heat energy. This occurs via enzymatic processes occurring first in the cytosol and then the mitochondria [4].

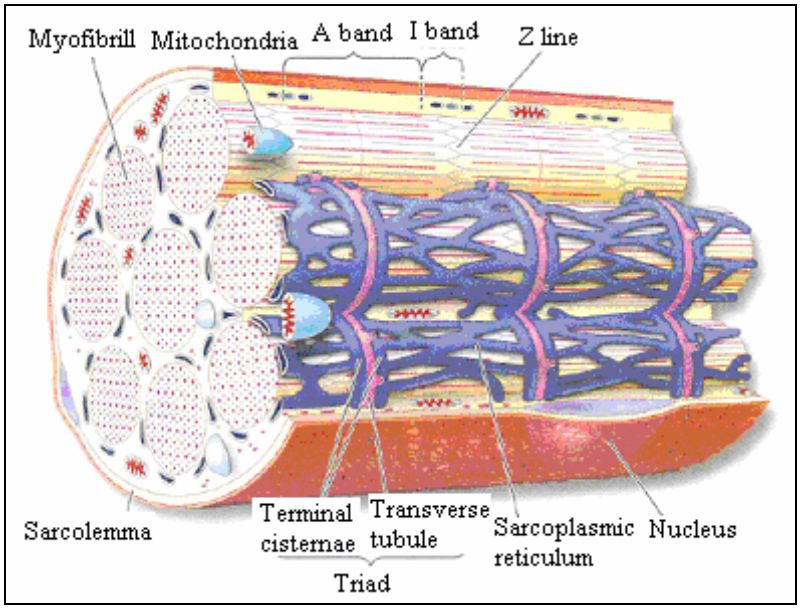

Figure 2.2 Cross section of a muscle fiber [4].

2.2 Muscle Fiber Types

Skeletal muscle does not simply contain a homogeneous group of fibers with similar metabolic and contractile properties. Skeletal muscle fibers can be classified into different groups on the basis of their speed of contraction and their resistance to fatigue upon repeated stimulation as slow-twich (type I) and fast-twich (type II). Type I fibers are only aerobic and contain an abundance of mitochondria [5, 6, 7].

Slow-twich fibers generate energy predominantly through aerobic pathways. Large, numerous mitochondria combined with high myoglobin levels give slow-twich fibers their characteristic red pigmentation.

A high concentration of mitochondrial enzymes links to a slow-twich fiber's enhanced metabolic machinery and makes them highly fatigue resistant and suitable for prolonged aerobic exercise. On the other hand they have low shortening speed [3, 4]. Fast-twitch fiber activities often demand rapid energy that only anaerobic pathways generate. These fibers have intrinsic speed of shortening and tension development three to five times faster than slow-twich fibers**.** This type has three subdivisions. Figure 2.3 shows

cross sections of a human muscle obtained by muscle biopsy with identification of type I and type II a, II b, and IIc fiber subdivisions.

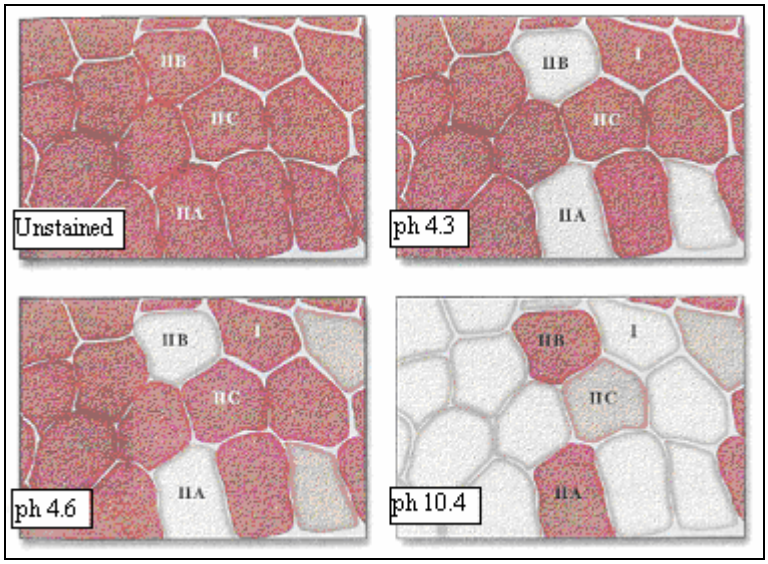

Figure 2.3 Serial cross sections of human vastus lateralis muscle obtained by muscle biopsy with identification of type I and type II a, II b, and IIc fiber subdivisions [4].

The intermediate type IIa fiber exhibits a fast shortening speed and a moderatly well-developed capacity for energy transfer from both aerobic and anaerobic sources. These fibers represent the fast-oxydative-glycloytic (FOG) fibers. Another subdivision, the type IIb fiber, possesses the greatest anaerobic potantial and most rapid shortening velocity, and represents the "true" fast-glycolytic fiber. The type IIc fiber, normally rare and undifferentiated, may contribute to reinnervation and motor unit transformation [3, 4].

Investigations on myosin isoforms have shown that rigorous exercise training results in alterations in muscle fiber types. However, training induced changes in fiber type are often small and do not result in a complete conversion. The transformation of a type IIb into a type IIa fiber is considered a fast to slow fiber shift, because the extent of an adaptive response is usually related to the magnitude of the inducing stimulus, the small changes in enzyme levels in fast fiber type relative to slow could reflect reduced participation in endurance exercise, the movement is from the fastest fiber type toward a slower fiber type [5].

In elite athletes, the relative percentage of fiber types appears to depend on whether the athlete's principal activity requires a short, explosive, maximal effort or involves sub-maximal endurance [8]. Knowledge of the composition and use of muscle fibers suggest that athletes with high percentage of ST fibers might have an advantage in prolonged endurance events, whereas those with a predominance of FT fibers could be better suited for short-term and explosive activities.

Table 2.1 summarizes characteristic properties of fiber types with respect to electrical activity patterns, morphological, histochemical, biochemical and function and contractility aspects.

Table 2.1
Classification schemes of skeletal muscle fiber types [4].

CHARACTERISTIC	FYBER TYPES		
	FAST - TWITCH		SLOW - TWITCH
	Type II b	Type II a	Type I
Morphology	**Fast Twitch b**	**Fast Twitch a**	**Slow Twitch**
Color	White	White/red	Red
Fiber diameter	Large	Intermediate	Small
Capillaries/mm^2	Low	Intermediate	High
Mitochondrial volume	Low	Intermediate	High
Histochemistry and biochemistry	**Fast Glycolitic**	**Fast Oxidative**	**Slow Oxidative**
Myosin ATPase	High	High	Low
Calcium capacity	High	Medium/high	Low
Glycloytic capacity	High	High	Low
Oxidative capacity	Low	Medium/high	High
Function and contractility	**Fast Fatigable**	**Fatigue Resistant**	**Slow Fatiguing**
Speed of action	Fast	Fast	Slow
Speed of relaxation	Fast	Fast	Slow

2.3 Mechanism of Muscle Contraction

Muscle contraction is the combination of electrical and mechanical events in a muscle fiber and start at the neuromuscular junction. The basic unit of contraction is a motor unit, composed of a group of muscle fibers and the somatic motor neuron that

controls them. When the somatic motor neuron fires an action potential, all muscle fibers in the motor unit contract. Figure 2.4 shows neuromuscular junction and a single alpha-motor unit of a skeletal muscle.

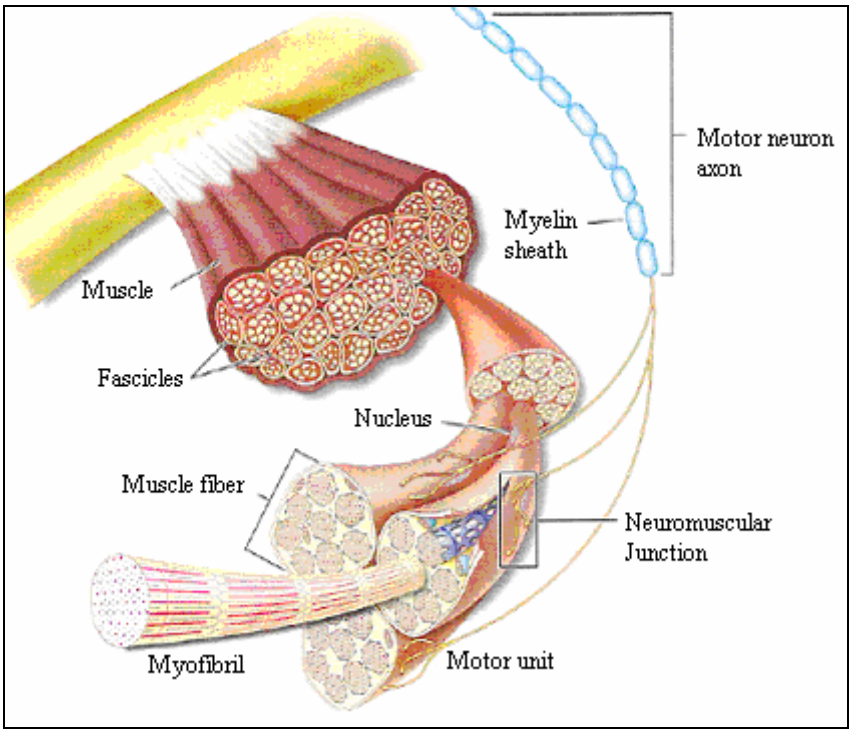

Figure 2.4 Neuromuscular junction and a single alpha-motor unit [9].

The electrical discharge at the muscle initiates chemical events at the cell suface, releasing intracellular Ca^{2+} and ultimately causing muscle action. Intracellular Ca^{2+} plays an intimate role in regulating a muscle fiber's contractile and metabolic activity. Ca^{2+} concentration within a nonactive muscle fiber remains relatively low compared with that of active muscle fiber. Muscle fiber stimulation causes an immediate, increase in intracellular Ca^{2+}, which precedes contractile activity. Cellular Ca^{2+} increases when the action potential at the transverse tubules causes Ca^{2+} release from the sarcoplasmic reticulum. The inhibitory action of troponin (which prevents actin-myosin interaction) rapidly dissipates when Ca^{2+} binds with this and other proteins in the actin filaments [4, 9].

Joining the active sites on actin and myosin, activates myosin ATPase to split ATP. The energy generated cause myosin crossbridge movement to produce muscle tension. The crossbridge uncouples from actin when ATP binds to the myosin crossbridge. Coupling

and uncoupling continues as long as Ca^{2+} concentration remains high enough to inhibit the troponin-tropomyosin system. This process is called excitation-contraction coupling. Figure 2.5 shows interaction between actin and myosin.

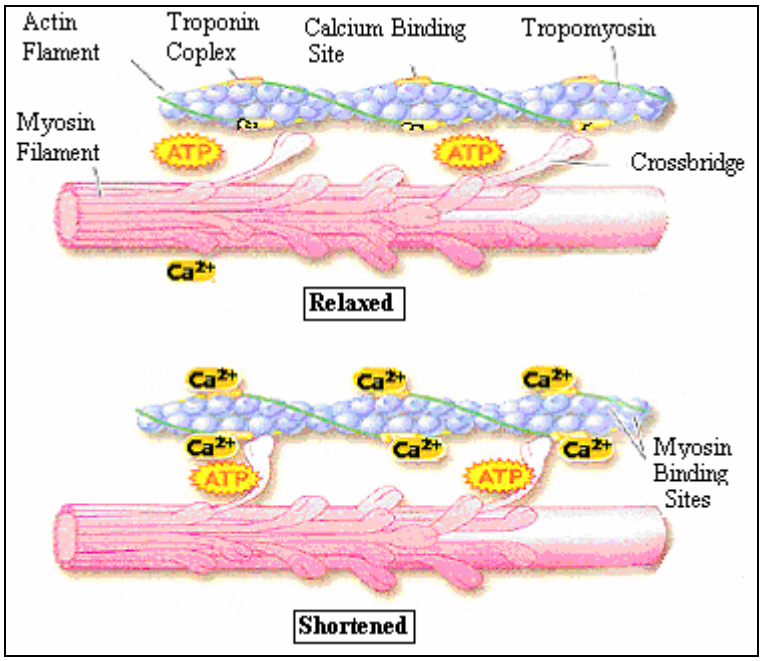

Figure 2.5 Interaction between actin and myosin. Ca^{+2} and ATP in relaxed and shortened muscle [4].

When muscle stimulation ceases, Ca^{2+} flow stops and troponin frees up to inhibit actin-myosin interaction. Recovery involves active pumping of Ca^{2+} into the sarcoplasmic reticulum. Retrieval of Ca^{2+} from the troponin-tropomyosin protein complex "turns off' the active sites on the actin filament and actin and myosin stay apart as long as ATP concentration remains adequate.

Deactivation serves two purposes: (i) it prevents any mechanical link between the myosin crossbridges and actin filaments and (ii) it inhibits the activity of myosin ATPase, which curtails ATP splitting. Muscle relaxation takes place when actin and myosin return to their original states [4, 9].

Figure 2.6 represents the structural arrangement of actin and myosin filaments during rest and contraction. Thick filaments pull on the actins toward the center by use of cross bridges. The sliding movement shortens the length of the entire sarcomere resulting

from the attachment of actin filaments to the Z-line. In a muscle fiber, the signal for contraction is synchronized over the entire fiber so that all of the myofibrils that make up the sarcomere shorten simultaneously. Figure 2.6 represents structural arrangement of actin and myosin filaments at rest and during contraction.

Motor units develop high, low or, intermediate tension in response to a single electrical stimulus. Additionally, motor units with low force capacity exhibit slow shortening but remain fatigue resistant, where as units with higher force capacity shorten rapidly but fatigue earlier. With prolonged aerobic training fast-twich muscle fibers become almost as fatigue resistant as slow-twich counterparts [4].

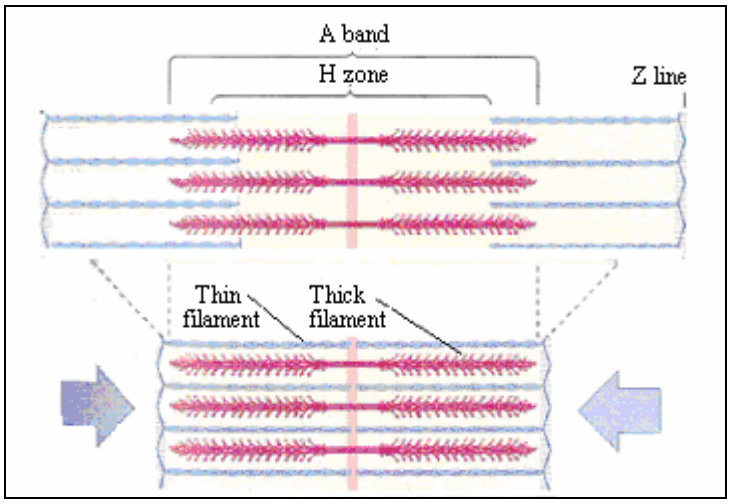

Figure 2.6 Structural arrangement of actin and myosin filaments at rest and during contraction [4].

2.4 Control Mechanism of Muscle Contraction

The brain combines two control mechanisms to regulate the force a single muscle produces. The first is motor unit recruitment and the second is rate coding.

The motor units that make up a muscle are not recruited in a random fashion and fire at the same time. From the stand point of neural control, the selective recruitment and firing pattern of fast-twich and slow-twich motor units provide the mechanism to produce the desired response [9].

Motor units are recruited according to the size principle. In accordance with the size principle, slow-twich motor units, with the lowest threshold for activation, become selectively recruited during light to moderate efford. These units are recruited first. As more force is demanded by an activity, progressively larger motor units are recruited. More rapid, powerful movements progressively type IIa units up through the type IIb units at peak force. Relatively large motor neurons with fast conduction velocities innervate the two major subdivisions of fast-twich muscle fibers [9]. Figure 2.7 shows speed, force and fatigue characteristics of motor units of fiber types.

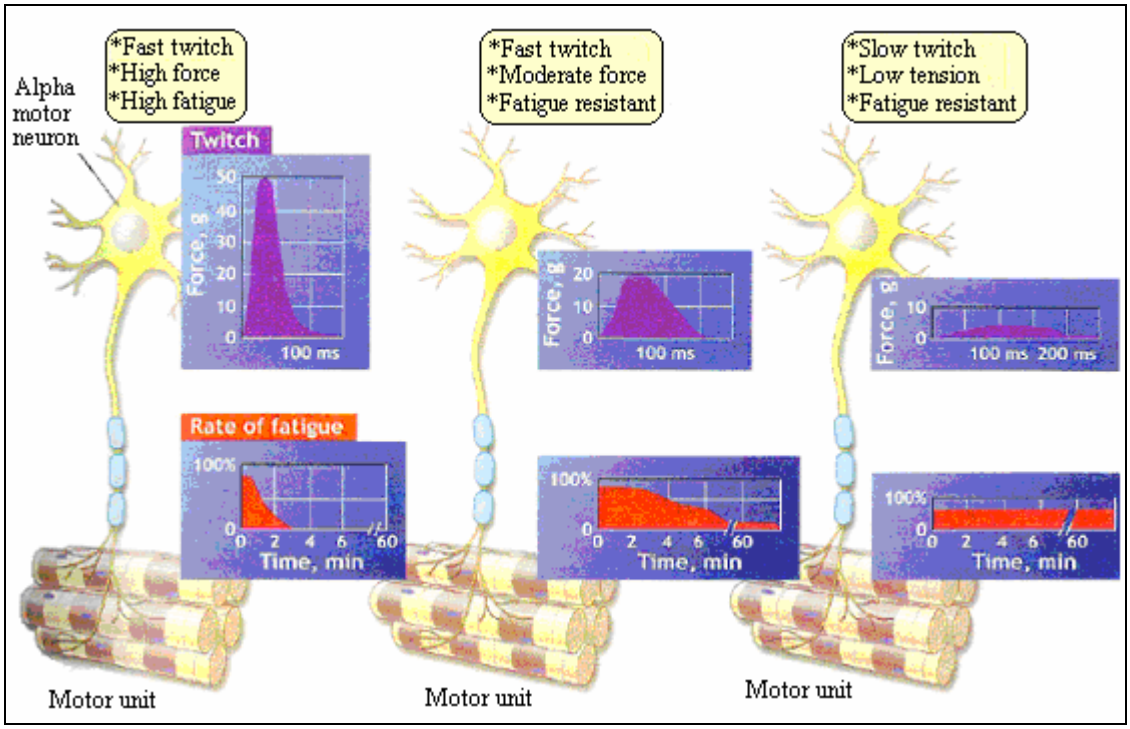

Figure 2.7 Speed, force and fatigue characteristics of motor units of fiber types [5].

Motor unit recruitment is regulated by required force. In the unfatigued muscle, a sufficient number of motor units will be recruited to supply the desired force. Initially desired force may be accomplished with little or no involvement of fast motor units. However, as slow units become fatigued and fail to produce force, fast units will be recruited as the brain attempts to maintain desired force production by recruiting more motor units. Consequently, the same force production in fatigued muscle will require a greater number of motor units. This additional recruitment brings in fast, fatiguable motor units.

There is a range of firing frequencies within a given motor unit. The motor unit's firing rate increases as the force of contraction exceeds its recruitment threshold. This is called rate coding. Slow units operate at a lower frequency range than faster units. Within that range, the force generated by a motor unit increases with increasing firing frequency. If an impulse reaches a muscle fiber before it has completely relaxed from a previous impulse, then force summation will occur. Thereby, firing frequency affects muscular force generated by each motor unit [4, 9].

2.6 Energy Utilization of Skeletal Muscle

ATP is the source of energy for all muscle contractions. A small amount exists in the muscle fiber cytoplasm when contraction begins. As ATP is transformed into adenosine diphosphate (ADP), another phosphate compound, phosphocreatine (PCr), transfers energy from its high-energy phosphate bond to ADP. The transfer replenishes the muscle's supply of ATP [4, 10, 11].

The combination of muscle ATP and phosphocreatine is adequate to support only about 15 seconds of intense exercise. Consequently, the muscle fiber must manufacture additional ATP from energy stored in the chemical bonds of nutrients. Some of these molecules are contained within the muscle fiber itself. Others must be mobilized from the liver and adipose tissue, afterwards transported to muscles through the circulation [4, 10].

The most efficient production of ATP occurs through aerobic pathways such as the glycolysis-citric acid cycle pathway. If the cell has adequate amounts of oxygen for oxidative phosphorylation, then both glucose and fatty acids can be metabolized to provide ATP. As the oxygen requirement of a muscle fiber exceeds its oxygen supply, energy production from fatty acids decreases dramatically and glucose metabolism shifts to anaerobic pathways. Anaerobic metabolism is also known as glycolytic metabolism, because in low oxygen conditions, anaerobic glycolysis is the primary pathway for ATP production. When a cell lacks oxygen for oxidative phosphorylation, the final product of glycolysis, pyruvate, is converted to lactic acid [10]. Figure 2.8 depicts energy metabolism in skeletal muscle.

Anaerobic metabolism has the advantage of speed, producing ATP 2.5 times as rapidly as aerobic pathways do. Howerver, anaerobic metabolism provides only two ATP per glucose, compared with the average of 30-32 ATP per glucose for oxidative metabolism, and it contributes to a state of metabolic acidosis by producing lactic acid. (However, the CO_2 generated during metabolism is a more significant source of acid.)[10].

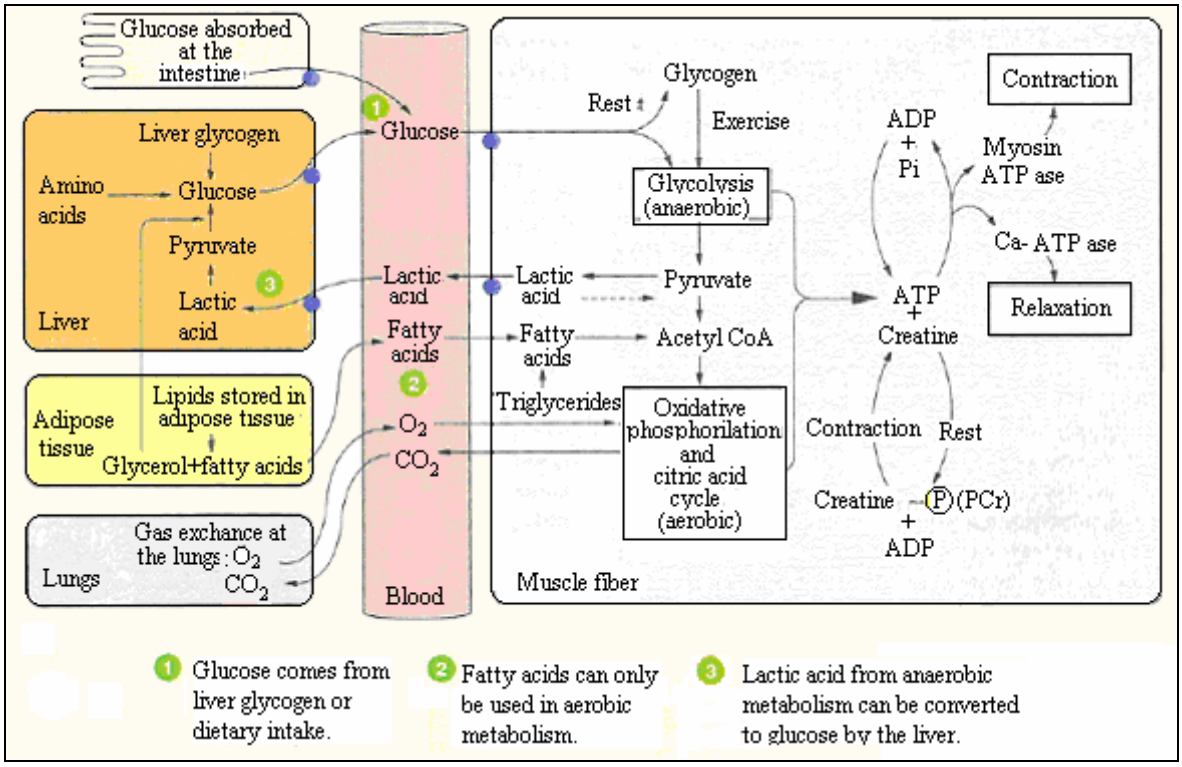

Figure 2.8 Energy metabolism in skeletal muscle [10].

Training induces altered energy utilization mechanisms to the muscle, providing evidence that the increase in the capacity to oxidize fat and carbohydrate is accompanied by a parallel rise in the capacity to generate ATP via oxidative phosphorylation [12, 13, 14].

Muscle glycogen concentration and total glycogen synthetase activity was increased in skeletal muscles of humans in response to exercise is generally elevated in trained individuals [15, 16, 17] suggesting that there is an increased capacity for glycogen synthesis in trained muscles. Additionally, serial biopsies during standardized submaximal exercise showed that men deplete their muscle glycogen stores less rapidly when they are

trained than when they are untrained [7, 18]. Also, intramuscular stores of triglycerides and capacity to hydrolyze triglycerides to free fatty acids may be increased by exercise-training appear to be increased in trained men [19].

Electron-microscopic studies on human skeletal muscles have provided evidence that training enables increases in both the size and number of mitochondria [5, 19]. Additionally, muscles of trained individuals appear to produce less lactate than those of untrained individuals, even at comparable rates of glycogenolysis [7].

2.7 Blood Flow and Oxygenation of Skeletal Muscle

Arteries and veins are oriented parallel to individual muscle fibers, and provide muscles with a rich vascular supply. Extensive branching of blood vessels, in and around the endomysium, assures each muscle fiber of an adequate supply of oxygenated blood from the arterial system and rapid removal of carbon dioxide in the venous circulation. An enhanced capillary microcirculation, which can be attained with intense aerobic training, expedites the removal of heat and metabolic byproducts from active tissues in addition to facilitating delivery of oxygen, nutrients and hormones. To accommodate the oxygen requirement during exercise, blood flow is redistributed as a result of a combination of vasodilation -which decreases peripheral resistance to blood flow- in skeletal muscle and vasoconstriction in other tissues [4, 10].

As muscles become active, changes in the microenvironment of the muscle tissue take place. Tissue O_2 concentrations decrease, where as temperature, CO_2, lactic acid in the interstitial fluid around the muscle fibers increase causing local vasodilation thereby shunting of blood flow from inactive tissues to the exercising muscles [5, 6].

When exercise commences, oxygen consumption increases rapidly, however this increase is not matched by the oxygen supply to the muscles. During the lag time, ATP is provided by muscle ATP reserves, phosphocreatine, and aerobic metabolism supported by oxygen stored on muscle myoglobin and blood hemoglobin [4, 10].

The use of these muscle stores creates an oxygen deficit because their replacement requires aerobic metabolism and oxygen uptake. When exercise stops, oxygen consumption slows down to resume its resting level. The excess post-exercise oxygen consumption (EPOC) formerly called the oxygen debt represents oxygen being used to metabolize lactate, restore ATP and phosphocreatine levels, and replenish the oxygen bound to myoglobin. Therefore blood flow distribution fluctuates in rhythmic activities. It decreases or becomes occluded locally according to exercise intensity during the muscle's contraction phase and increases during relaxation to provide a "milking action" that moves blood through the muscles and propels it back to the heart. The rapid dilation of previously dormant capillaries complements the pulsatile blood flow [10].

Reactive compensations become superimposed on the feedforward changes as exercise proceeds. Muscle chemoreceptors sense the buildup of lactic acid and metabalites and send this information along to central command centers in the brain. The command centers maintain changes in ventilation and circulation that were initiated in a feedforward manner [10].

Studies demonstrated an increased capillary density in skeletal muscle in trained subjects [20, 21, 22], and oxygen extraction fraction was significantly higher in the exercising muscle in athletes. In addition, blood transit time -the time blood stays in capillaries- in the exercising muscle was longer thereby potentially allowing more efficient oxygen extraction and perfusion was more homogeneous in the exercising muscle in the trained subjects [23].

Also, higher peripheral arteriovenous oxygen difference has previously been found in trained compared with untrained legs providing ease in oxyganation to the exercising muscle[24, 25, 27]. Additionally, flow heterogenity which may be a reason for reduced O_2 transfer efficiency and O_2 diffusing capacity, was found to be less due to vascular adaptation in the trained compared with the untrained subjects during exercise [23, 26].

2.8 Fatigue

The term fatigue describes a condition in which a muscle is no longer able to generate or sustain the expected power output. Fatigue is highly variable. It is influenced by the intensity and duration of the contractile activity, by whether the muscle fiber is using aerobic or anaerobic metabolism, by the composition of the muscle, and by the fitness level of the individual.

Multiple factors have been proposed to play a role in fatigue. They can be classified into central fatigue mechanisms that arise in the central nervous system, and peripheral fatigue mechanisms that arise anywhere between the neuromuscular junction and the contractile elements of the muscle.

Central fatigue includes subjective feelings of tiredness and desire to cease activity. Several studies have shown that this fatigue actually precedes physiological fatigue in the muscles and therefore may be a protective mechanism.

Lactic acid production from anaerobic metabolism is often mentioned as a possible cause for fatigue. Same argument suggests that acidosis caused by lactic acid dumped into the blood may influence the sensation of fatigue perceived by the brain. However, because the homeostatic mechanisms for pH balance maintain blood pH at normal levels until exertion is nearly maximal, pH is not a factor in central fatigue in most instances of submaximal exertion [3].

Neural causes of fatigue could arise either from communication failure at the neuromuscular junction or from failure of the central nervous system command neurons. For example, if acetylcholine, which initiates the sequence of muscle membrane events leading to contraction, is not synthesized in the axon terminal fast enough to keep up with the firing rate of the neuron, neurotransmitter release at the synapse will decrease. Consequently, the muscle end-plate potential may fail to reach the threshold value needed to trigger a muscle fiber action potential, resulting in contraction failure. This type of

fatigue is associated with same neuromuscular diseases, but it is probably not a factor in normal exercise. Fatigue within the muscle fiber itself can occur in any one of several sites.

The most plausible factor that cause the difference between the static and dynamic contractions is the blood flow in the contracting muscle. The intramuscular pressure during the static contraction prevents the blood flow, and the metabolic byproducts, such as lactic acid, accumulate in the muscle. The blood flow may change the intramuscular pH or the concentration of potassium ion (K^+). These ions influence the excitability of the muscle fiber membrane and affect muscle fiber conduction velocity (MFCV) [28, 29]. During maximal exercise, K^+ leaves the cell with each action potential, and K^+ concentrations rise in the extracellular fluid of the t-tubules. The shift in K^+ alters the membrane potential of the muscle fiber and is believed to decrease Ca^{2+} release from the sarcoplasmic reticulum [30]. The conduction of excitation along the muscle fiber is affected by the gradient of potentials across the muscle fiber membrane. The serum K^+ concentration increases during sustained muscle contraction [31] because the blood flow in a muscle is prevented by the contraction. Then the gradient across the muscle fiber membrane decreases and the excitability of the muscle fiber membrane also decreases. The change of Na^+/ K^+ conductance becomes slower [32] and the muscle fiber conduction velocity decreases. During dynamic contraction the blood flow removes K^+ from the contracting muscle. Consequently, the excitability of the muscle fiber membrane is not inhibited and MFCV does not decrease [29].

A new theory suggests that elevated phosphate decreases Ca^{2+} release because the phosphate combines with Ca^{2+} to become calcium phosphate. However, some investigators feel that phosphate alters the dynamics of Ca^{2+} release from the sarcoplasmic reticulum [33, 34, 35, 36].

Also, the role of H^+ in muscle fatigue has sometimes been considered. H^+ was observed to be related to the decline in force. Miller et al. (1988) observed a linear relationship between H^+ and fatigue [37, 38, 39].

Zwarts and Arendt-Nielsen [40] reported that the peripheral occlusion occurs in a contracting muscle during an isometric contraction with a load of 50% MVC or greater

[29]. Under ischemia, oxygen supply to an active muscle is prevented, and lactic acid rapidly accumulates due to enhanced glycolytic metabolism. The accumulation of lactic acid decreases pH in a contracting muscle. This also inhibits the excitability of the muscle membrane [40, 41]. The produced H^+ is consumed chemically and metabolically in the muscles. The buffering system by bicarbonate ($HC0_3^-$) has a significant role in the homeostasis of the pH. This buffering system of $HC0_3^-$ depends on the intracellular pressure of CO_2 ($PC0_2$). Because intracellular $PC0_2$ increases under the ischemic condition, this buffering system can not work effectively during the prolonged static contraction [29, 42].

On the other hand, during dynamic contraction, oxygen supply is maintained through the blood flow, and the buffering system of $HC0_3^-$ works effectively because an excessive increase in $PC0_2$ does not occur [42]. Furthermore, a decrease in pH can be inhibited by the removal of H^+ with the blood flow. Therefore, intramuscular pH and the excitability of the muscle membrane are stable during dynamic contraction, and consequently, MFCV does not decrease with time [29].

In extended submaximal exertion, fatigue is associated with the depletion of glycogen stores within the muscle. Most studies show that lack of ATP is not a limiting factor, glycogen depletion may be affeding some other aspect of contraction [43, 44].

There appears to be no single cause for muscle fatigue during exercise, and the study of this phenomenon is quite complex.

2.9 Types of Muscle Contractions

Muscle contractions can be classified mainly according to the relationship between either the muscle tension and the resistance to be overcome or the muscle moment generated and the resistance to be overcome.

2.9.1 Isometric Contractions

During an isometric contraction, no joint motion occurs. The limitation of isometric contractions is that the muscle is only strengthened at the specific joint angle that is used in the exercise. As a result, this type of muscle contraction is typically used for specialized purposes [45].

2.9.2 Concentric Contractions

Both concentric and eccentric contractions involve joint motion. Concentric contractions require the greatest energy expenditure, followed by isometric and eccentric contractions. Concentric contractions involve what is termed "positive work" the muscle is shortened while developing tension [45].

2.9.3 Eccentric Contractions

Conversely, eccentric contractions occur when the muscle is lengthened while developing tension. Eccentric contractions usually occur when a muscle acts to oppose the force of gravity. Eccentric contractions generally develop greater muscle tension than both isometric and concentric contractions. Subsequently, the muscle is also more vulnerable to rupture during an eccentric contraction [45].

3. MUSCULAR ENDURANCE TESTING

Muscular endurance is the ability of a muscle or muscle group to execute repeated dynamic contractions or to statically maintain a specific percentage of MVC (Maximum Voluntary Contraction) for a prolonged period of time till fatigue. Muscular endurance tests are performed to predict future performance, indicate weaknesses, measure improvement, assess the success of the training programme, place the athlete in appropriate training group, and motivate the athlete.

Muscular endurance may be measured using a fixed percentage of a person's body weight, a fixed percentage of a one's one RM (repetition maximum), an absolute weight, or calisthenic-type exercises. However, good norms have yet to be established with most of these types of test protocols. In addition, since there is a broad variation in exercise equipment design, it would be difficult to establish comparison norms for individuals who test on the different equipment [46].

3.1 Fixed Percentage of a Person’s Body Weight Method

Initially the subject’s body weight is determined. Then, the amount of resistance for each lift by multiplying the body weight by the percentage of each lift is calculated [46].

3.2 Fixed Percentage of One RM Method

With the fixed percentage of one’s one RM method, usually 70 per cent of the subject‘s one RM is preferable. Again, due to a lack of established norms, the number of successful lifts may be used as a marker to assess gains in muscular endurance [46].

3.3 Absolute Method

With the absolute method, muscular endurance is assessed by counting the number of successful repetitions of a "fixed" load over time [47]. The disadvantage of this method is that it does not take into account a person's body weight [48].

3.4 Calisthenic-Type Exercises

Muscular endurance tests using calisthenic-type exercises are convenient to use. The curl-up and push-up are the most common calisthenic-type muscular endurance tests used by fitness professionals. The curl-up (for abdominal muscle endurance) has been suggested as an alternative to the sit-up due to dissatisfaction with the involvement of the hip flexors when performing a sit-up motion [47]. Test is terminated when the subject can not maintain the cadence or performs the curl-up with improper technique. The purpose of push-up test is to measure upper body muscular endurance. The standard and modified push-ups are often used [49].

3.5 Assessment Equipments: Free Weights and Resistance Machines

Methods of assessing muscular fitness are based on factors which include muscle groups to be tested, availability of equipment and cost. Free weights and various types of resistance machines are most commonly used for muscular strength and endurance assessment. Free weight exercises employ important stabilizing muscles and requires more motor coordination and balance resulting in greater muscle recruitment, compared with machines which do not emphasize the stabilizing musculature because movements occur in only one plane of motion. Free weights also give freedom to test different exercise variations compared to resistance machines. Although, resistance machines offer a convenient, safe and easy way to test muscular fitness [49, 50] however, it is difficult to evaluate a single muscle by this way.

There are several examples of utilization of endurance tests in various investigations on different muscle or muscle groups. Such as Motebe et al. evaluated Tr (Recovery Time), grip strength, exercise endurance time and forearm circumference, and made comparative investigations. Dynamic hand grip exercise was carried out at %30 MVC, one repetition per second, until the point of exhaustion. The time to exhaustion was recorded as the endurance measurement [51].

Another investigation, from Coronell et al., evaluated quadriceps endurance (contractions against a load equivalent to %10 MVC until task failure or for up to a limiting time of 30 minutes, in their reaserch about assessing quadriceps endurance in patients with COPD (Cronic Obstructive Pulmonary Disease) [52].

On the other hand, Wilson et al. conducted a more broad investigation and utilized 7 different endurance tests in their research targeting formation of trunk and lower extremity muscle endurance normative data for adults. Utilized tests were included for the upper abdominals (sit up); upper trunk extensors (chest raise); lower abdominals (supine, double straight leg raise); lower trunk extensors (prone, double straight leg raise); and the half squat. All tests were performed statically - just one repetition - until fatigue set in [53].

4. NEAR-INFRA RED SPECTROSCOPY (NIRS)

The diagnostic potential of optical methods has been widely known since Jöbsis [54] first demonstrated that transmittance measurements of near-infrared (NIR) radiation could be used to monitor the degree of oxygenation of certain metabolites. However, the use of optical methods, exploiting visible light (400-650 nm) to investigate muscle oxidative metabolism, dates back to 1937, when Millikan (Millikan 1937) demonstrated muscle deoxygenation on stimulation, its application is limited by the poor penetration depth. Conversely, near-infrared (650-1100 nm; NIR) spectroscopy (NIRS), a relatively new non-invasive technique, allows the simultaneous measurement of changes in intravascular and mitochondrial oxygenation in 2-6 cm of the limb muscles [55].

This led to the development and increasingly widespread use of clinical near-infrared spectroscopy (NIRS), which offers a safe, non-invasive means of monitoring cerebral and muscle function at the bedside without the use of radioisotopes or other contrast agents [54]. Various NIR optical techniques, differing in the type of light source (lamps, lasers, light-emitting diodes) and in the modality of the light sources (continuous wave, NIR_{CWS}; continuous wave spatially resolved, $NIRS_{SRS}$; pulsed, NIR_{TRS}; phase modulation, NIR_{PMS}) have been used for muscle studies [55].

With the advent of near-infrared spectroscopy (NIRS), the clinical significance of NIRS muscle findings in different diseases has been explored by several groups using different instrumentation. NIRS has been used to investigate the diseases associated with impaired tissue oxygenation, like heart failure and peripheral vascular disease (PVD) [55].

It has been shown that patients with congestive heart failure desaturate their muscle at lower work levels than normal subjects indicating an insufficient blood flow to the exercising muscles of these patients (Wilson et al. 1989; Mancini et al. 1994; Belardinelli et al. 1995; Matsui et al. 1995). The results indicate that NIRS can detect different muscle oxygenation profiles in patients with different levels of exercise intolerance [55].

Kovacs and collegues' study is an example in clinic investigations of NIRS in which oxygen use of the erector spinae muscle was explored to determine whether oxygen use differed between healthy and low back pain (LBP) individuals. They monitored oxygen use and blood volume in the lumbar region via NIRS. Results showed significant differences in oxygen use but not blood volume between healthy and LBP subjects with muscular-based disorders. They concluded that inability of the muscular group to use oxygen in a manner similar to the healthy group indicates different processes at the tissue level, indicating that differences in oxygen use may provide insight into why motion patterns differ between healthy and LBP groups [56].

To clarify whether oxygenation of peripheral muscle is one determinant of exercise tolerance, Takashi et al. simultaneously examined the oxygen uptake kinetics and oxygen kinetics of peripheral skeletal muscle by NIRS during recovery from exercise in COPD patients. Fifteen patients with cronic obstructive pulmonary disease (COPD) and five normal control subjects compared using NIRS for changes in concentration of oxygenated hemoglobin (HBO_2) and during expired gas analysis. They found that the time constant of oxygen uptake during recovery from constant work rate exercise and the time constant of HBO_2 during recovery were significantly longer in COPD patients than in normal control subjects and claimed that oxygen uptake kinetics during recovery is related to re-oxygenation of peripheral skeletal muscle evaluated by NIRS in patients with COPD. Therefore, NIRS may be a useful tool to estimate the impairment of cardiopulmonary responses and re-oxygenation of peripheral skeletal muscle during the immediate recovery phase after exercise in COPD patients [57].

Kemp et al. applied ^{31}P-MRS/NIRS/MRI to calf muscle of dialysed patients to define the abnormalities in contractile efficiency, mitochondrial function and vascular O_2 supply during isometric plantarflexion and recovery in 23 male patients. They noticed that slowing of post-exercise phosphocreatine (PCr) recovery implied a defect in effective mitochondrial capacity. That PCr recovery was slow relative to NIRS recovery suggests that this is largely an intrinsic mitochondrial problem not the result of impaired O_2 supply [58].

In another study from Kemp et al., to study ATP synthesis and oxygenation in calf muscles of normal subjects and patients with peripheral vascular disease ^{31}P magnetic resonance spectroscopy with near-infrared spectroscopy (NIRS) is combined. They proposed an analysis of interactions between metabolic 'error signals' and cellular PO_2 which is estimated from NIRS changes, provisionally assumed to reflect deoxymyoglobin. In patients the inferred 'mitochondrial capacity' is decreased by %53-%60, leading to reduced oxidative ATP contribution in exercise, because of increased deoxygenation. Increased PCr perturbation partially outweighs cellular hypoxia, but as low cellular PO_2 is required for capillary-mitochondrion O_2 diffusion, rate-signal relationships may overstate maximum oxidative ATP synthesis rate [59].

Many studies were made to investigate peripheral vascular disease (PVD). Cheatle et al. (1991) found that at rest the O_2 consumption of PVD patients were half of that found in healthy controls. A standardized treadmill exercise was used to investigate the calf oxygenation of patients with claudicatio intermittent (Komiyama et al. 1994; McCully et al. 1994a; Colier et al. 1995a). The patients with more severe impairment (i.e. insufficient O_2 delivery) presented an earlier decrease of muscle oxygenation. A delayed oxygenation recovery time after forearm occlusion was found in patients with chronic subclavian artery occlusion (Kurosawa et al.1996) [55].

Macnab et al. investigated a study to determine whether near infrared spectroscopic monitoring of the spinal cord is feasible, and whether changes in near infrared spectroscopy correlate with changes in blood flow to the cord or operative maneuvers and hypothesized that near infrared spectroscopy could be used to monitor the spinal cord's cellular energy equilibrium during spinal surgery that vascular compromise could be identified before irreversible damage occurred due to capability of NIRS for measuring absolute changes in the concentration of three chromophores: oxygenated hemoglobin, deoxygenated hemoglobin, and cytochrome aa3, the terminal enzyme in the electron transfer chain and a measure of cellular energy equilibrium. Resuls showed that with reduced oxygen delivery (lower arterial oxygen saturation and blood flow), oxygenated hemoglobin concentration decreased and deoxygenated hemoglobin concentration increased. With distraction, blood volume (oxygenated hemoglobin plus deoxygenated hemoglobin) decreased, and cytochrome aa3 became more oxidized. Changes were

apparent within 1 second of the intervention beginning, and recovery to the baseline of near infrared spectroscopy occurred with relief of each intervention. They concluded that near infrared spectroscopy technique monitors changes in oxygenation of the spinal cord, and therefore appears to be capable of intraoperative warning about impending vascular compromise of the spinal cord [1].

According to Chance & Bank oxidative defects have been found in metabolic myopathies by NIR_{CWS} (Bank&Chance 1994; Chance & Bank 1995). Additioanlly McCully & Posner claimed that ageing might be associated with muscle blood flow reduction. It was found that elderly subjects had a longer rate of calf O_2 resaturation (Mc Cully & Posner 1995) [55].

The results indicate that NIRS can detect different muscle oxygenation profiles in patients with different levels of exercise intolerance. As well, scientists have successfully used this noninvasive technique to examine changes in skeletal muscle respiration during a variety of exercise conditions including intermittent, continuous isometric, concentric and eccentric muscular contractions [2].

In the Eighties several NIR_{CWS} prototypes were built (Jöbsis 1977; Giannini et al. 1982; Takada et al. 1987; Cope & Delpy 1988; Chance et al. 1988*b*; Hampson & Piantadosi 1988); forearm occlusion and exercise was used to test their instrumental capabilities [55].

The majority of exercise studies using NIRS have been descriptive to ascertain that this technique can be used to examine alterations in muscle deoxygenation and blood volume [61] during short-term acute exercise. Mancini et al. have shown NIRS to be a valid [62, 63, 74] and reliable [64, 60] technique for measuring relative changes in muscle deoxygenation and blood volume.

NIRS has found a number of applications in the study of hemodynamic and oxygenation-related parameters in tissue and exercise induced changes in muscle metabolism. Such as, quadriceps oxygenation was investigated during constant and incremental work rate bicycle exercise (Wilson et al. 1989; Chance et al. 1992; Belardinelli

et al. 1995; Costes et al. 1996). It has been demonstrated that the rate of O_2 resaturation after exercise is faster in the endurance trained athlete (rowers) than in sedentary controls (Chance et al. 1992) [55].

Costes et al. suggested that endurance training improves the oxygen delivery and muscle metabolism. In the study muscle oxygen saturation measured by near infrared spectroscopy which is primarily influenced by the local delivery/demand balance, should thus be modified by training. Exercise tests were performed before and after a four-week endurance-training program. The result was training-induced adaptation in blood lactate influences SO_2 (oxygen saturation) during mild- to hard-intensity exercise. Thus, NIRS could be used as a noninvasive monitoring of training-induced adaptations [65].

Takaishi et al. examined circulatory and metabolic changes in a working muscle during a crank cycle in a pedaling exercise with NIRS by sampling measurements under stable metabolic and cadence conditions during incremental pedaling exercise according to the crank angles whose signals were obtained in eight male subjects. The changes in muscle blood volume during a crank cycle demonstrated a pattern change that corresponded to changes in pedal force and electrical muscle activity for pedal thrust. Significant differences in the level of NIRS parameters (muscle blood volume and oxygenation level) among work intensities were noted to be related to pedal force and a temporary increase in muscle blood volume following a pedal thrust was detected at work intensities higher than moderate. It was found that changes in muscle blood volume might reflect muscle blood flow restriction caused by pedal thrusts and NIRS parameters serves as a useful measure in providing additional findings of circulatory dynamics and metabolic changes in a working muscle [60].

In another study of Takaishi et al. the effects of cycling experience and pedal cadence on the NIRS parameters among noncyclists, triathletes, and cyclists at a work intensity of %75 maximal O_2 uptake while changing pedal cadence was investigated. Physiological and biomechanical responses and NIRS parameters were measured**.** The bottom peak level in muscle blood volume of the NIRS changes and the difference in levels between the peak of the temporary increase and the bottom peak of reordered NIRS change determined for blood volume are available to detect noninvasively the differences

in circulatory dynamics and metabolic change during pedaling exercises performed at different pedal cadences and also to estimate the difference of physiological and technical developments for endurance cycling in athletes [61].

Binzoni et al. found that ischemia-reperfusion cycles do not induce metabolic changes but alter the interstitial fluid readjustment in their study on energy metabolism and interstitial fluid displacement in the human gastrocnemius during three subsequent 5-min ischemia-reperfusion periods. The muscle energy balance was assessed by combining near-infrared spectroscopy (NIRS) and ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-NMRS**)** and the interstitial fluid displacement was determined by combining NIRS and ^{23}Na-NMRS [62].

Neary et al. demonstrated that the metabolic properties of different fiber types are altered with exercise, and the improvements related to changes in metabolic properties of the muscle by examining short-term endurance training changes in terms of cardiorespiratory responses and muscle deoxygenation trends. Eight male cyclists performed an incremental cycle ergometer test to voluntary exhaustion, and a simulated 20-km time trial on wind-loaded rollers before and after training. NIRS was used to evaluate the trend in vastus medialis hemoglobin/myoglobin deoxygenation (Hb/Mb-O_2) during both tests pre- and post-training. The results showed a significant improvement in maximal oxygen consumption induced by short-term endurance training was due primarily to central adaptations, whereas the simulated 20- km time trial performance was enhanced due to localized changes in muscle oxygenation [63, 66].

Kouzaki et al. investigeted the relation between local circulation and alternate muscle activity among knee extensor synergists during low-level sustained knee extension at two and a half per cent of maximal voluntary contraction in rectus femoris (RF) and vastus lateralis (VL) by using near-infrared spectroscopy and poined out that local circulation is modulated by the alternate muscle activity of knee extensor synergists, and a negative correlation between the muscle activity and blood volume sequences was found in only RF but not in VL [59].

According to Bhambhani et al. the use of NIRS as an alternative noninvasive method for detecting ventilatory threshold during cycle exercise in healthy subjects concidering the onset of anaerobic (lactate) metabolism during incremental exercise, which may be a result of an imbalance between tissue oxygen supply and demand, has been associated with the gas exchange ventilatory threshold [64] and found that the rate of decline in recovery for a given increase in peak oxygen uptake was independent of exercise mode and gender [67].

Oguri et al. examined the effect of training at moderate altitude (1800m) which is often used by athletes to stimulate muscle hypoxia and near sea level using near infrared continuous wave spectroscopy (NIRcws). During the exercise, the changes in oxygenated hemoglobin (HBO) in the vastus lateralis muscle, arterial oxygen saturation, and heart rate were measured using NIRcws and pulse oximetry, respectively. They reached a conclusion that moderate altitudes might be effectively used to apply hypoxic stress on peripheral muscles [68].

Ding et al. evaluated whether NIRS can be used to assess metabolic capacity of muscles with an exercise on a cycle ergometer which was performed by 18 elite male athletes and eight healthy young men from the quadriceps muscle at two wavelengths (760 and 850 nm) in order to calculate relative index of muscle oxygenation until exhaustion. During exercise, changes in muscle oxygenation and blood lactate concentration were recorded and oxygen saturation and the relative value of the effective decrease in muscle oxygenation examined. According to results they concluded that oxygen saturation and the relative value of the effective decrease in muscle oxygenation may be distinctive variables that can be used to characterise muscle oxidative metabolism during human body movement [69].

Kalliokoski et al. found that endurance-trained subjects have a higher oxygen extraction fraction in the exercising muscles, which could potentially be associated with the observed longer blood transit time and more homogeneous perfusion. These changes could contribute to the enhanced oxygen supply to muscle cells and improved exercise efficiency in the endurance-trained subjects [23].

In human experiments, heterogeneity of deoxygenation level among the quadriceps muscle has been found and muscle fiber types, capillary density, and muscle recruitment have been suggested as the reasons [72]. Quaresima et al. applied the venous occlusion 10 seconds after the start of the exercise when tHb (total hemoglobin) was stable- not to measure muscle blood flow (MBF)- because, as expected, the intramuscular pressure was high enough to restrict completely MBF to the investigated muscle group. The contraction intensity was at about %70 of the subject's own maximal voluntary contraction. Therefore, the venous occlusion applied while, high intensity isometric contraction was maintained, should not affect the perfusion of the contracting muscle, the performance of the exercise, and the muscle metabolic response to the exercise. MBF heterogeneity which can be attributed to divergence of the mechanical activity within the quadriceps, differences in oxidative metabolic activity, variations in the vascularization, i.e., distribution of arterial, venous and capillary vessels; and the recruitment of different fiber types within the investigated muscle volume was found in the quadriceps during dynamic and isometric exercise. In conclusion, frequency-domain oximeters are found to be relatively low-cost, noninvasive, powerful tools for investigating the spatial and temporal features of the muscle oxygenation changes as well as the MBF in pathophysiology [70].

It has been shown that NIRS can play a significant role in understanding healthy muscle oxidative metabolism, understanding the effects of disease on muscle metabolism and function, evaluating the efficacy of therapeutic intervention; and confirmation of disease diagnoses.

4.1 fNIRS (Functional Near Infra-Red) Device

Human tissues contain a variety of substances whose absorption spectra at NIR wavelengths are well defined, and which are present in sufficient quantities to contribute significant attenuation to measurements of transmitted light. The concentration of some absorbers, such as water, melanin, and bilirubin, remain virtually constant with time. However, some absorbing compounds, such as oxygenated hemoglobin (HbO_2), deoxyhaemoglobin (Hb), and oxidised cytochrome oxidase (CtOx), have concentrations in tissue which are strongly linked to tissue oxygenation and metabolism. NIRS is based on

differential absorption properties hemoglobin and myoglobin (Hb/Mb) in the near-infrared range of 700-1000 nm. At 760 nm. deoxygenated Hb/Mb has a higher absorbency and at 850 nm. oxygenated Hb/Mb has a higher absorbency [73, 74]. Therefore, the difference in reflected signal between the two wawelenghts indicates the degree and trend of deoxygenation at the level of arterioles capillaries and venules [75]. Figure 4.1 shows absorption properties of hemoglobin and myoglobin (Hb/Mb) in the near infrared spectrum.

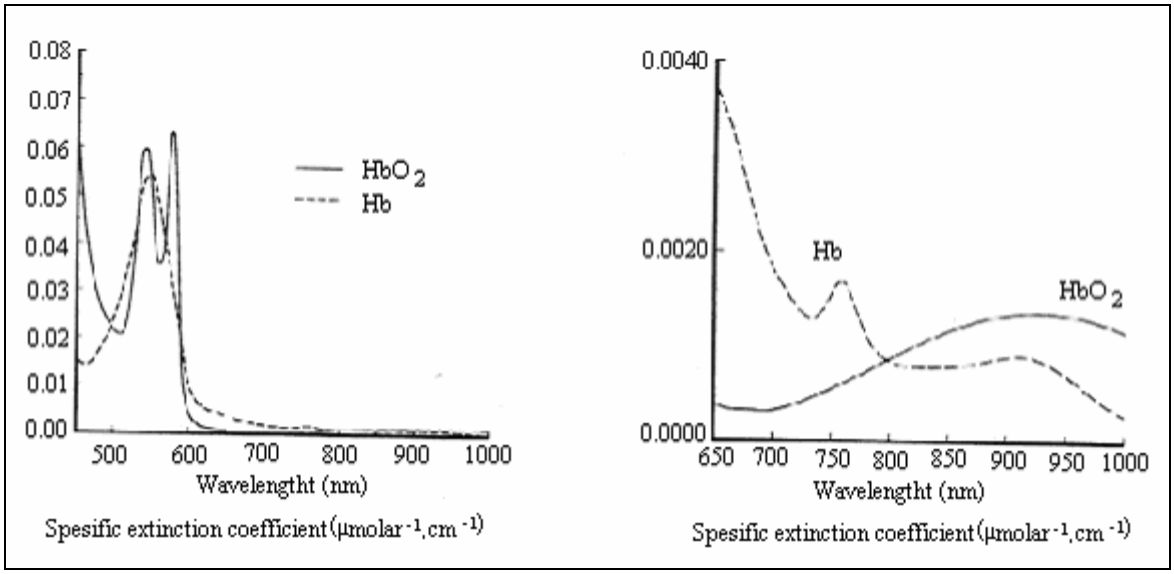

Figure 4.1 Absorption properties of hemoglobin and myoglobin (Hb/Mb) in the near infrared spectrum [54].

With the 805 nm wavelength region -the region of equal absorption for oxygenated and de-oxygenated Hb- deoxygenated Hb and Mb cause an increase in absorption in the shorter wavelengths, while oxygenated Hb and Mb cause an increase in absorption in the longer wavelengths. It thus follows that light from the emitter of 735 nm. in wavelength is more easily taken up by de-oxygenated Hb and Mb, while light of 850 nm. in wavelength is more easily taken up by oxygenated Hb and Mb. The amount of light that then returns to the detectors can be measured and in this way it is possible to measure approximately the oxygenation and de-oxygenation states of Hb and Mb. The mean penetration depth of NIRS in living tissue is approximately one-half of the distance between the emitter and detector [76, 77].

The device is composed of probe and main computer body, and the probe is equipped with emitter and detectors for near-infrared light of 735 nm., 805 nm. and 850 nm. wave-lengths. The emitted light passes through the skin and, while scattering, reaches tissue where a portion is absorbed by Hb and Mb and then returns to the detectors. Figure 4.2 represents a schematic describtion of fNIRS device and Figure 4.3 shows fNIRS device (NIROXCOPE 201) developed at the Biophotonics Laboratory in Bogazici University.

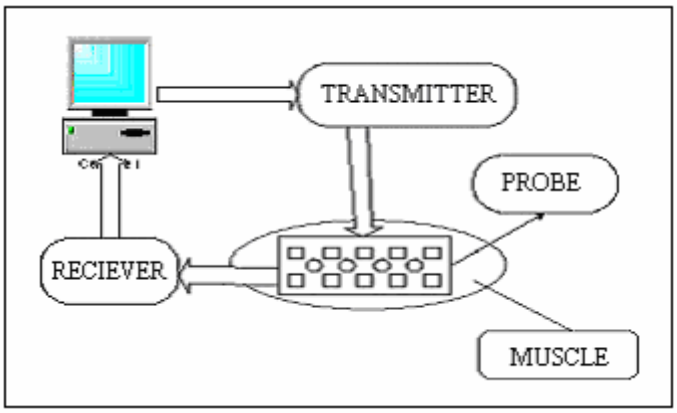

Figure 4.2 fNIRS device.

Human muscle performance and oxygen consumption evaluation techniques (fMRI, blood oxygenation level dependent (BOLD) contrast MRI, H-NMRS, P-NMRS and 31P MRS) are being utilized to increase our knowledge about the muscle performance and metabolic processes [78]. However, these techniques are expensive, physically constraining, and confine the participants to restricted positions. These disadvantages give preferance to fNIRS due to ease in measurement, noninvasive use, application at the tissue level, and during short periods of ischemia, gives information about tissue oxyhemoglobin and deoxyhemoglobin concentration changes. The latter changes, in the absence of inflow and outflow to and from the tissue, reflect the functional changes induced by oxidative metabolism [78]. It is portable, relatively inexpensive, and negligibly invasive, and can be used repeatedly with the same participants. This makes fNIRS suitable for the study of hemodynamic changes under many in situ working conditions (e.g., computer operation), in sensitive paradigms, in studies requiring repeated session, and among infants and children.

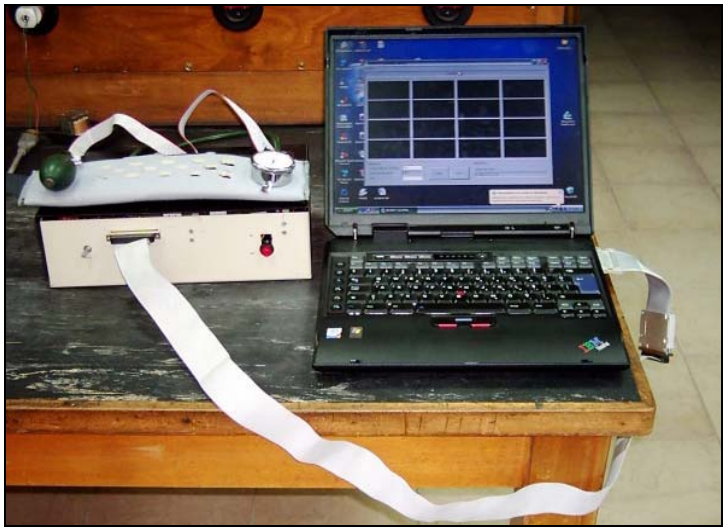

Figure 4.3 fNIRS device (NIROXCOPE 201) developed at the Biophotonics Laboratory in Bogazici University

5. SURFACE EMG (ELECTROMYOGRAPHY)

Electromyography records changes in electrical potential of a muscle when it is caused to contract by a motor nerve impulse, often are used to study muscle functions. Changes in EMG measures may indirectly indicate the development of muscle fatigue which is most commonly described as occurring when there is a decrement in the amount of tension a muscle can generate, or when the muscle can no longer maintain the required force level due to exercise.

Surface and intra-muscular electrodes have been used mainly to detect and analyze myoelectric activities of individual muscles. Studies indicate a gross variation between individuals when needle electrodes are used because of different depths of muscle [79]. Surface EMG (sEMG) measurements is an excellent method for obtaining summated activity, can be used during maximum voluntary contraction, can be repeated as often as necessary and carries no risk of cross-infection and surface electrodes provide good co-operation of young children with the absence of needles. Therefore, surface electrodes have several advantages, including noninvasiveness, easy to adhere to the skin and to detect the total activities of the muscle. They have been widely used to investigate neuromuseular functions of the extremities and or body trunk of healthy subjects as well as patients and is well-suited to applications in kinesiology and fatigue research, where longer lasting or repeated measurement is often required [80].

Exercise and sport physiologists also routinely use sEMG [80, 81, 82]. Most studies of neuromuseular activity and fatigue have evaluated isometric contractions [8, 83, 84, 85]. The analysis of myoelectric signal detected under stationary conditions have not yet been thoroughly explored clinically.

In fatigue studies, the mean frequency (MNF) of the power density spectrum is regarded as a useful parameter. During fatiguing contractions the MNF shifts to lower frequencies. It is supposed that the changes of the MNF during fatigue are caused by a

combination of central factors (variations in motor neuron firing patterns) and peripheral factors (variations in the muscle fiber itself, especially the MFCV).

Additionally, the integrated EMG (iEMG) data shows values that are increased for a longer period of time (hours) after fatiguing contractions at moderate force levels which suggests a decrease in muscular efficiency [20].

The increase in EMG amplitude has been suggested to reflect fatigue induced increases in motor unit recruitment and/or firing rate [87] while the decrease in median power frequency may reflect decreases in muscle fiber conduction velocity [86, 88] however, the mechanisms underlying these effects are still under investigation.

Root Mean Square (RMS), Mean Frequency (MNF) and Median Frequency (MDF) is commonly used to describe signal energy and frequency content of the signal, respectively. The behavior of the surface EMG during various protocols of fatigue has been extensively investigated generally during static contractions [89, 90, 91]. It has been proposed that the RMS and MDF provide information relative to the number and location of the active motor units, the recruitment of motor units, the shape of the motor unit action potentials, the mean firing rate of the individual motor units, and the extent of superposition of action potentials from concurrently active motor units [90]. Figure 5.1 shows an idealized version of the frequency spectrum of the EMG signals.

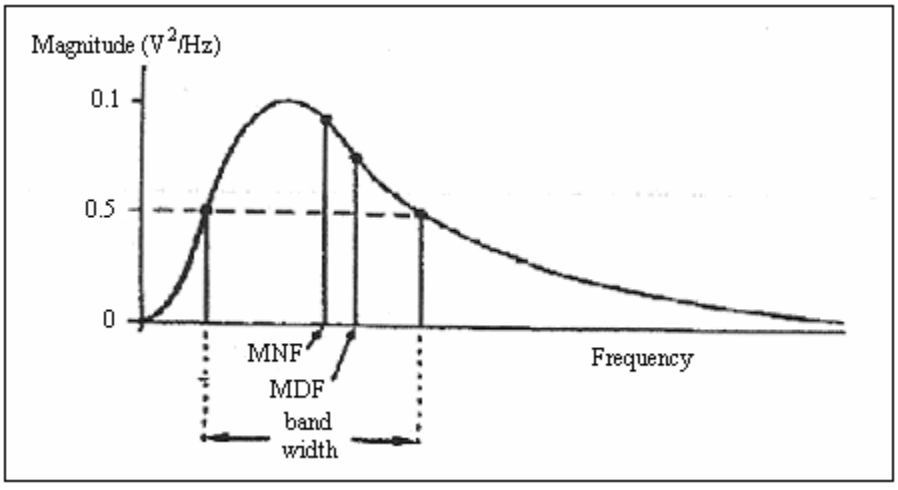

Figure 5.1 An idealized version of the frequency spectrum of the EMG signals [95].

The majority of the literature states that MDF [92, 93, 94] and MNF [36] are the most reliable spectral parameters in monitoring changes in the muscle fiber propagation, muscle temperature, muscle fatigue and muscle fiber type. In addition to this, researches demonstrated that the power spectrum of the EMG signal was found to shift toward the lower band during prolonged muscle contraction when the localized muscular fatigue occurs.

Hagberg investigated the endurance time for sustained isometric exercise and dynamic exercises measured at different contraction levels in nine male volunteers. There were significant differences between the regressions of endurance time vs. the contraction levels for the sustained isometric exercise. The development of muscle fatigue was well correlated to changes of the myoelectric RMS amplitude however, the MNF differences in exercise did not significantly effect the relation between the time constant of the MNF decrease and the endurance time [96].

Kankaanpaa et al. developed a submaximal repetitive isoinertial back muscle endurance test in order to define the relationships between the power spectral indices of paraspinal muscle electromyographic activities, endurance time and a subjective estimate of fatigue. It is concluded that MNF and MPF measured before the onset of total muscle fatigue are good predictors of endurance time and are closely related to the subjective perception of fatigue [97].

In Gerdle and Karlsson's study, the shift in the MNF of the power density spectrum of the EMG during endurance tests of the knee extensors were investigated until exhaustion. This study demonstrated the use of MNF as a valid indicator of peripheral fatigue [98].

Another study evaluting muscular fatigue is executed by Hary et al. EMG and bandlimited noise signals of comparable spectra were processed to compute the centroid and median frequencies and the high/low ratio for different record lengths, signal amplitudes, and noise bandwidths**.** This relationship made the results of that study applicable to a wide variety of EMG tests of endurance [99].

Gabriel et al. investigated the neural mechanisms responsible for the increase in strength during serial isometric contractions. A three-session design was used. Subjects were asked to perform five maximal isometric elbow extension strength trials to serve as baseline. After a 5-min rest, the subjects were administered a 30-trial fatigue protocol. This process was repeated two more times at two weeks intervals. Elbow extension torque and sEMG of the triceps and biceps brachii were monitored concurrently. The criterion measures were elbow extension torque, RMS EMG amplitude, and MPF. Findings suggest that the fatigue protocol served as a training stimulus to down regulate motor-unit firing frequency [100].

It is known that endurance training is accompanied by neuromuscular adaptations that would alter the production and/or clearance of metabolic substrates. Walton et al. tried to determine the extent of reflex inhibition during and after fatigue in endurance-trained individuals compared to sedentary controls. Subjects produced isometric ankle plantarflexion contractions at %30 of maximal voluntary contraction (MVC) until their MVC torque declined by %30. The endurance-trained subjects showed a greater reduction in H-reflex amplitude early in the fatiguing protocol compared to the sedentary subjects. These experiments have demonstrated that the neuromuscular processes associated with fatigue-related reflex inhibition must be multi-faceted and cannot be explained solely by small-diameter afferents responding to the byproducts of muscle contraction [101].

Gerdle et al. investigated the relationship between muscle morphology and surface electromyographic parameters, MNF and RMS, during sustained static knee extension to exhaustion at %25 MVC and at %70 MVC. The endurance time increased with decreasing force level. A significantly higher perception of fatigue was found at %25 MVC than at %70 MVC. From principal component analyses it was concluded that RMS at %25 MVC mainly correlated with the type II muscle fibre proportions (per cent), and at %70 MVC mainly with the areas of type II muscle fibre. At %25 MVC, MNF correlated with the areas of type IIa, IIb and IIc fibres, and at %70 MVC negatively with the proportion of type IIb and to some extent with areas of type IIa, IIb and IIc fibres. The study indicated relationships between surface EMG and muscle morphology [102].

In the last ten years, the complexity of the sEMG has led to the development of a multiplicity of measurement protocols and processing methods. Based on theoretical models, and understanding of the information carried by this signal requires a description of its characteristics using several parameters. This characteristic is supposed to provide useful information about neuromuscular function.

6. INVESTIGATIONAL METHODS AND PROCEDURES

6.1 Subjects

Participants are male adults between the ages of 20 and 27, with no reported history of neurological or orthopedic disorder and have different physical condition status. They participated in the study voluntarily and gave their informed consent before the experimental procedure. Demographic data of subjects are presented in Table 6.1 and statistical values are given in Table 6.2.

Table 6.1
Subjects' demographic data

No	Subject Initials	Age	Weight (kg)	Height (m)	Body Mass Index (B.M.I)	Exercise Frequency
1	A.K	25	75	1,73	25,06	6 / month
2	K.S	22	75	1,81	22,89	4 / week
3	L.T	27	95	1,84	28,06	1 / month
4	M.H	20	72	1,79	22,47	rarely
5	M.E	20	68	1,80	20,99	1 / week
6	M.P	20	63	1,76	20,34	3 / week
7	M.A	21	72	1,75	23,51	rarely
8	Ö.Ç	25	80	1,80	24,69	6 / month
9	Sa.K	26	98	1,90	27,15	50 / month
10	Se.K	25	77	1,75	25,14	1 / week
11	U.A	21	67	1,75	21,88	2 / month
12	U.E	27	72	1,83	21,49	rarely

Table 6.2
Statistical values of demographic data

Subject	Number	Minimum	Maximum	Mean	Standard Deviation
Age	12	20	27	23,2	2,83
Weight	12	63	98	76,5	10,9
Height	12	1,73	1,90	1,79	0,05
B.M.I	12	20,3	28	23,3	2,37

This study was conducted at the Biophotonics Laboratory in Biomedical Engineering İnstitute of Boğaziçi University .Before the test, the subjects were given details concerning the protocol of the test.

The subject was seated upright in a specially constructed exercise chair which permitted adjustment for differences in height and leg lenght to enable maintenance of constant body position during contraction.

6.2 Method

The exercise model was chosen for three reason. First, it is a well-controlled model in which the rate of work can be regulated easily. Second, it is a pure isometric model, and third, it avoids motion artifacts and enables application of two different methods at the same time. A variety of muscular endurance assessment strategies are available, yet isometric endurance testing seems to be cost-effective and requires less equipment for testing [103]. Squat wall endurance test for the evaluation of quadriceps muscle group is found to be safe due to being a closed chain exercise (CKC) for anatomical components of knee joint and suitable to use prolonged periods of time [104].

Another factor that makes squat endurance test adventageous is that isometrically contracting muscle, which is held for an extended period of time, causes blood flow to be either compromised or occluded because of the high intramuscular pressure. Depending on the anatomical relationship of muscle and bone, blood flow may be occluded at forces only slightly higher than %15 MVC. When the muscle is deprived of its capability of supply and removal it becomes a closed system which makes NIRS measurement possible without applying occulusion.

Each electrode site was shaved, abraded, and cleaned with alcohol to facilitate electrode adherence and conduction of EMG signals. Adhesive silver-silver chloride (Ag-AgCl) surface electrodes, having a diameter of 0.5 cm, were placed over the belly of the right vastus lateralis muscle with a 2.5 cm inter electrode distance.

Electrode placement was determined by placing subjects in the appropriate test positions and a thigh girth measurement was taken at a point 50 per cent of the distance between the inguinal ligament and the superior pole of the patella of the rigt leg. The electrode was placed lateral to an imaginary line bisecting the anterior superior iliac spine and the superior patellar pole at the 50 per cent position described above. The lateral distance equaled 15% of the thigh girth measurement and electrode placed after controlling the muscle bellies by palpation [106]. All electrodes were positioned parallel to the muscle fibers. A common ground electrode was placed laterally on the lateral epicondyl of tibia. fNIRS probe was placed just below the surface EMG electrodesas represented in Figure 6.1. Elastic wraps were applied to maintain electrode and probe placement during the test. All test sites were identified and prepared by the same researcher.

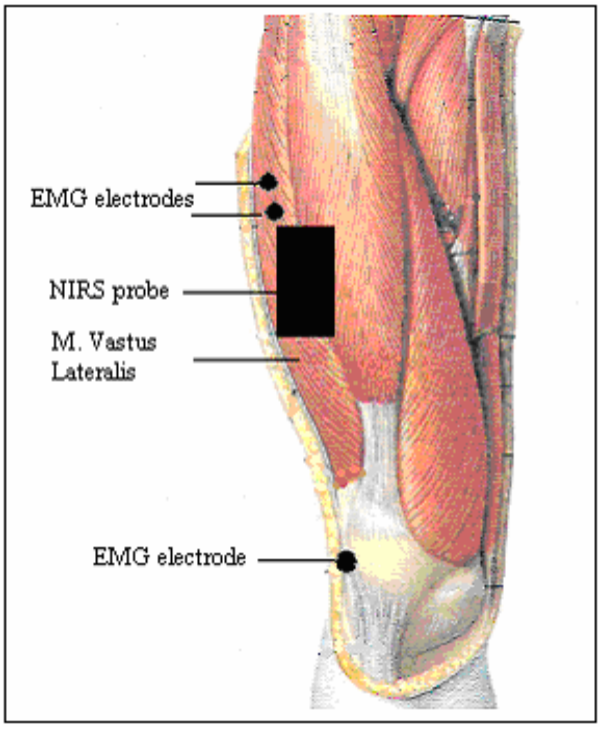

Figure 6.1 Location of EMG electrode and NIRS probe placement on vastus lateralis (VL) muscle selected in the experiments [105].

Two tests were conducted separated by an interval of five minutes. The first test involved maximum voluntary isometric contraction of quariceps muscle group enabling optimum contraction of vastus lateralis with the foot was in full dorsi flexion and eversion for ten seconds. After the break the second test was performed in chair squat or modified

half squat and lasted untill subject experienced the feeling of fatigue. Duration of squat exercise varied among subjects and experiments ended according to subjects' own decision or when they were unable to maintain the spesific knee angle determined at the onset of the trial.

Figure 6.2 represents determination of maximum voluntary isometric contraction (MVIC) while subject was sitting and the leg was in full extention with the foot was in total dorsi flexion and evertion. This section is ended in 10 seconds while subject was having continious verbal feedback to keep the contraction in maximum steady state from the conductor.

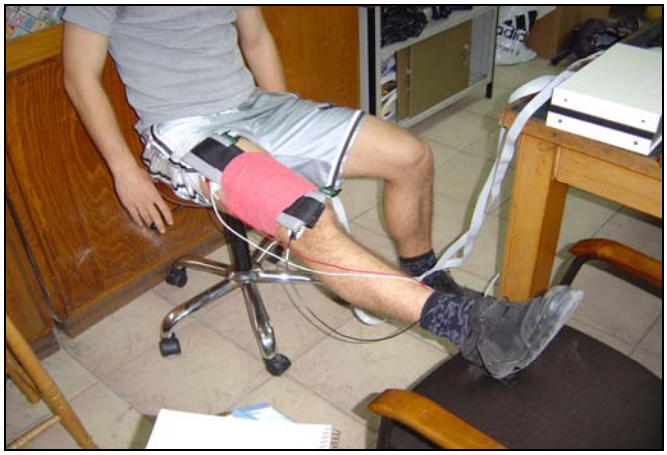

Figure 6.2 Measurement of maximum voluntary isometric contraction (MVIC) for 10 seconds.

MVIC measurements were used to determine percentage of VIC (Voluntary Isometric Contraction) during the squat exercise for each subject from raw EMG amplitude values.

For the squat exercise, participants sit in a comfortable chair without back support providing knee angle 70 degree flexion from full extention. Knee angle determination was supplied using a manual goniometer. After having the subject in appropriate positon EMG electrodes and NIRS probe attached on Vastus Lateralis of the Quadriceps muscle. Initially baseline measurement was taken for one minute. Figure 6.3 shows the subject's position during baseline measurements and Figure 6.4 shows squat exercise.

Then participants took the wall-sit position providing knee flexion 110 degree, with the back of the participant completely leaning to the wall and keep their position up to fatigue. Lastly participants return to baseline position again for recovery time measurements for five minutes. All tests conducted by same person and were ended according to subject's decision.

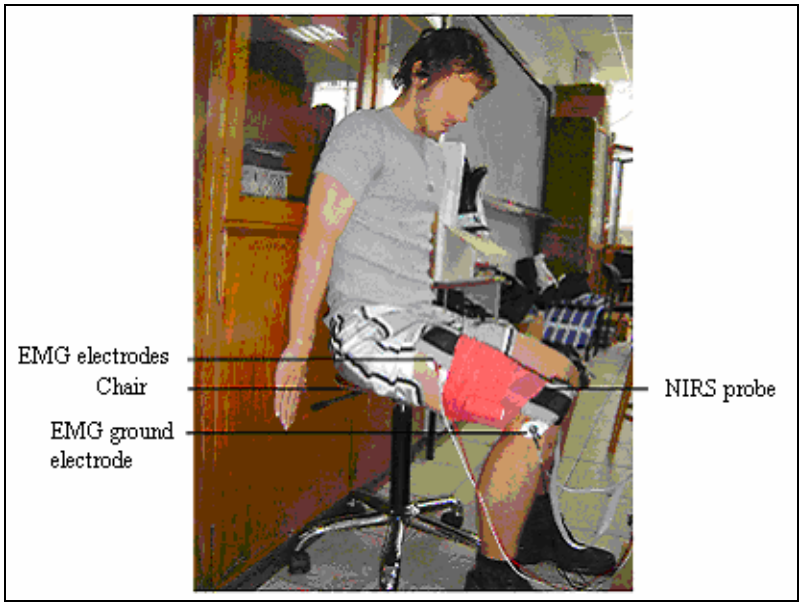

Figure 6.3 Baseline measurement prior to the squat exercise.

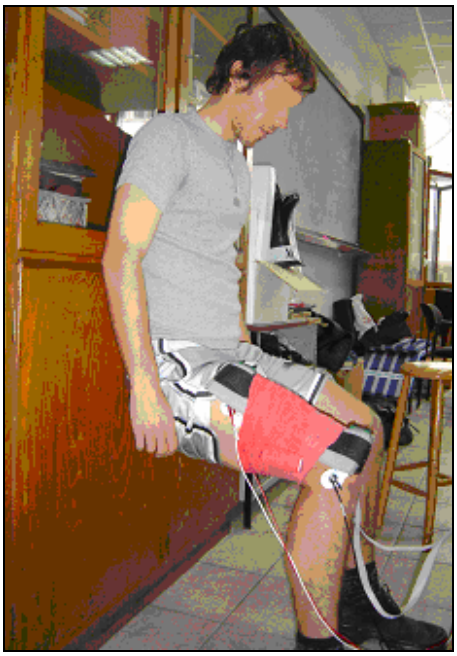

Figure 6.4 Measurements during squat exercise until fatigue.

Both fNIRS and EMG data were gathered during the experiments. Data from fNIRS were used to examine the O_2 kinetics during exaustive endurance exercise and sEMG data was evaluated in order to distinguish subjective feeling of fatigue and physiological fatigue between two groups. Additionally both techniques evaluated whether a relationship exists between O_2 consumption characteristics and EMG signals.

6.3 Instrumentation

The fNIRS system was developed at the Biophotonics Laboratory (NIROXCOPE 201) and houses a probe that contains four LED light sources (Epitex L4X730/4X805/4X850- 40Q96 multi-wavelength LED) each emitting at three near infrared wavelengths and ten photodetectors (TI-Burr Brown, OPT101) that when time and wavelength-multiplexed end up with four non-overlapping quadruples of photodetectors. The detectors are placed equidistantly at 2.5 cm away from the center of a source within each quadrant. Taking into consideration Firbank and et al.'s study [107], a pre-determined source detector separation of 2.5 cm accounts for an average adult cortex depth around 1.5 cm that allowed us to probe the muscle in interest. The probe is positioned such that its base aligns with direction of vastus lateralis muscle. However, placement of sEMG electrodes partially restricted the placement of the probe. Because vastus lateralis is a longitudinal, narrow and oblique muscle, data from only one detector was suitable for evaluation of measurements.

While HB recovery half times and slopes, and HBO_2 and OXY decline amplitudes and slopes were determined as an indicator of endurance performance, BV (Blood Volume) measurements are not evaluated. Data from detector four evaluated in all experiments because of its location. Figure 6.5 and 6.6 shows a schematic describtion fNIRS parameters.

Raw EMG signals were monitored with surface electrodes (Ag-AgCl). Signals were acquired with BIOPAC Model MP30 hardware and BIOPAC Student Lab. Pro Software systems. It is well known that the bandwidth of the surface EMG signals (sEMG) is less than 1000 Hz. The EMG signals were therefore sampled at a rate of 2000 Hz.

System passed signals between 30-1000Hz. Notch filter was used to remove power line interference at 50 Hz. During data acquisition data were passed to hard disc for storage.

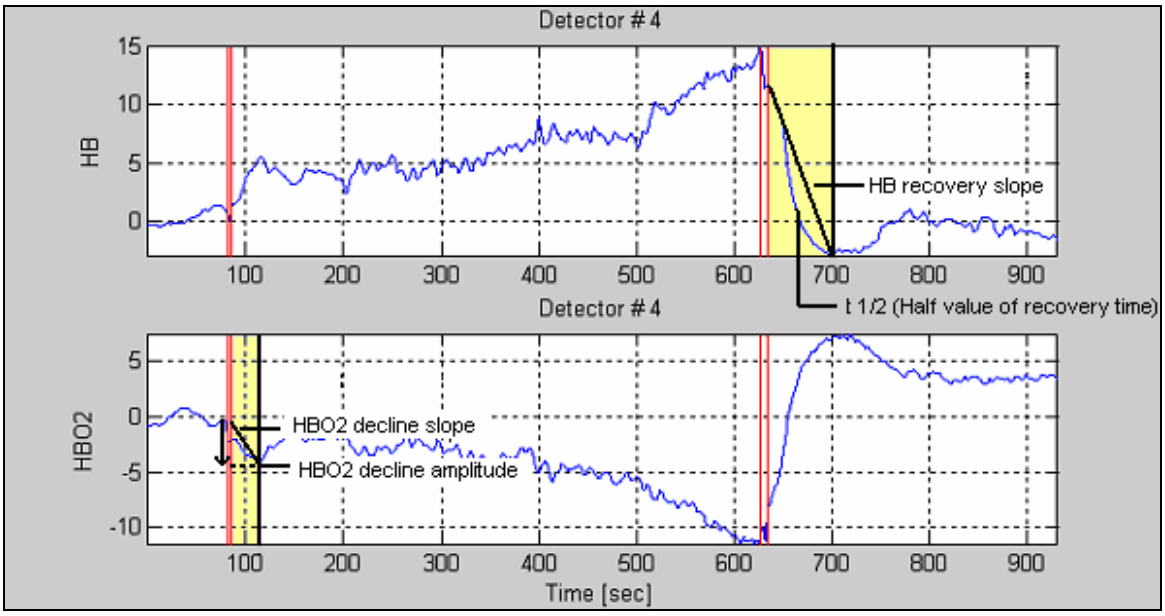

Figure 6.5 Schematic describtion of fNIRS HB (Deoxyhemoglobin) and HBO2 (Oxyhemoglobin) parameters.

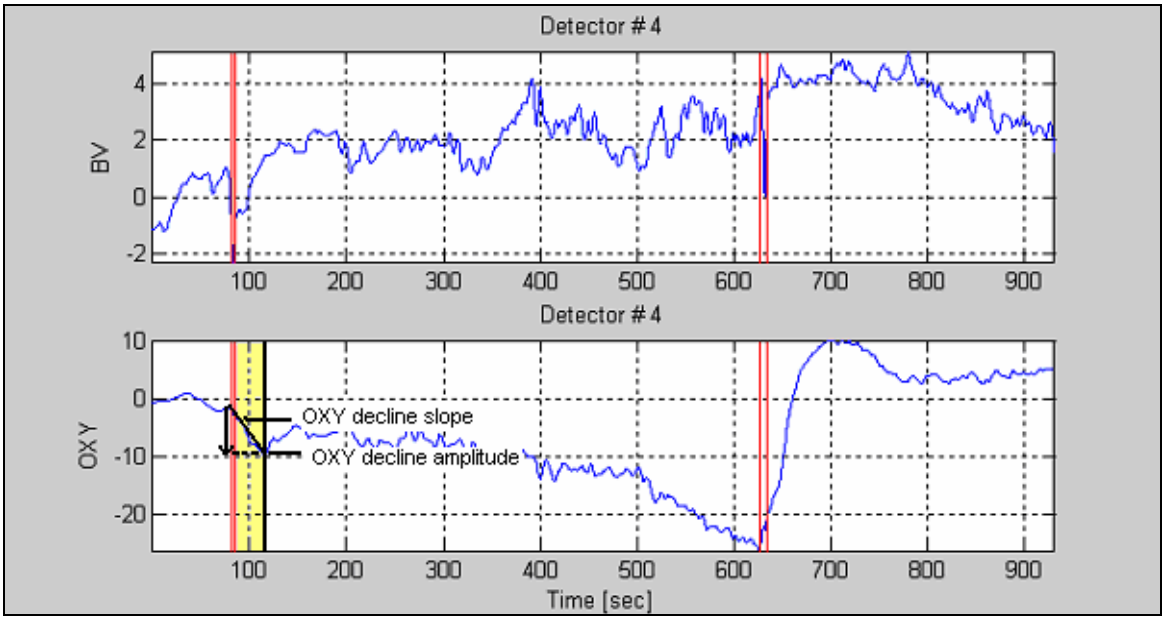

Figure 6.6 Schematic describtion of fNIRS OXY (| HBO2 | - | HB |) parameter.

Data analysis was performed off-line using MATLAB 6.5 (Release 13) software. The power spectrum was derived from the raw EMG signal using the Fast Fourier Transform (FFT) algorithm. The median frequency (MDF) and the mean frequency (MNF) of the power spectrum were computed from the EMG signal with consecutive 0.5 seconds windows (non-overlapping). This duration was chosen in order to remain in a wide-sense stationary region of the EMG signal and to get enough data for the analysis in each window. Root mean square (RMS) at each sample are calculated as the root of the average of the sum of square of sEMG values in the neighbourhood of 0.25 sec (500 samples for 2000 Hz sampling rate). From the measurements raw EMG data is examined and RMS, MNF and MDF slopes were optained.

7. RESULTS

The subjects were groupped into two according to the duration of squat exercise they performed as; the subjects maintaining squat position more than five minutes (Group I) or less than five minutes (Group II). Figure 7.1 and 7.2 represents a typical measurement sample from NIRS data for Group I, and Figure 7.3 and 7.4 represents a typical measurement sample from NIRS data for Group II.

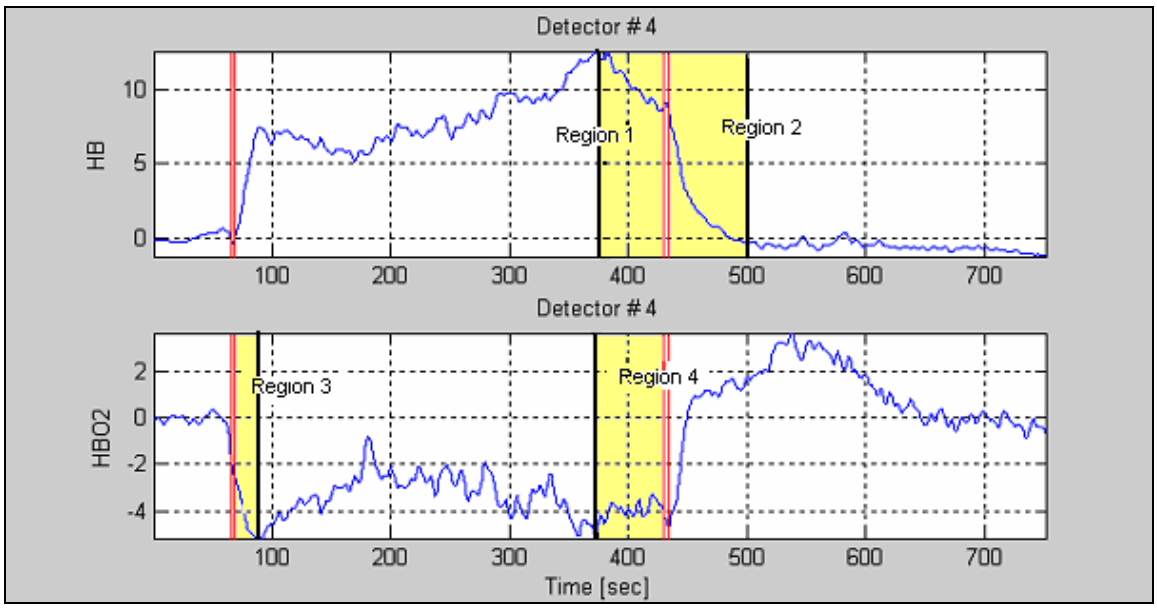

Figure 7.1 A typical sample indicating HB and HBO2 parameters measurement belong to a subject from Group I.

Above data represents HB and HBO2 signal features of a regularly exercising subject. HB recovery slope and t1/2 parameters obtained from region one where the decrease in HB is sharp and reach a minimum point. For group I, slope value is expected to be bigger and t1/2 is smaller than group II due to training induced adaptations such as, elevated mitochondrial density, and enhanced activity of oxidative enzymes, augmented the arterio venous O_2 difference as a result of training [66]. In this subject recovery slope is 0,1377 and t1/2 is 33,8 secods.

Regarding HBO_2 signal, in region three, initial decline slope and amplitude were expected to be bigger as a result of enhanced aerobic metabolism due to training. Also, several studies have shown an increased capillary-to-fiber ratio in skeletal muscle after endurance training [20, 21, 22], which could potentially affect flow heterogeneity resulting in reduced O_2 transfer efficiency and O_2-diffusing capacity [26] and impair oxygen extraction by the muscles. These parameters' values are found to be as 0,1775 and 3,3 micromolar respectively.

Additionally, in region one and four, decrease in HB and increase in HBO2 may indicate a reduction in intramuscular pressure due to transfer of body weight to to other leg rather than equal distribution of the whole weight between two extremities consequently a reduction in occlusion. However, this may as well be due to insufficiency of maintaining knee angle at desired level and involving additional muscles to the exercise leading to a decrease in the degree of contraction level.

Moreover, the rise in HBO2 signal in the area between region three and four may indicate again a decrease in occlusion steming from altered cardiovascular performance or the subject may be leaned the other leg leading to a decrease in the degree of contraction level in the measured leg. Another explanation for this situation may be: intramuscular pressure forces cellular serum to interstitial region causing an increase in diffusion area and prevents to some extent, the transport of metabolites, such as O_2, to the tissue in spite of vasodilation [4] and increased heart rate and blood pressure.

The data in figure 7.2 shows the initial decline slope and amplitude value of OXY signal, in region one, which are found to be 0, 4880 and 10,5 micromolar respectively and were expected to have the same behaviour with HBO2 parameters because of higher O_2 extraction [23] and cappilarization status [66].

Similiarly, in region two, a trend to increase in OXY signal is seen happening at the same time with HBO2 increase and HB decrease due to decrease in occlusion. Moreover, the signal characterictics in the area between region one and two supports the idea about HBO2 behaviour in figure 7.1 the area between region three and four.

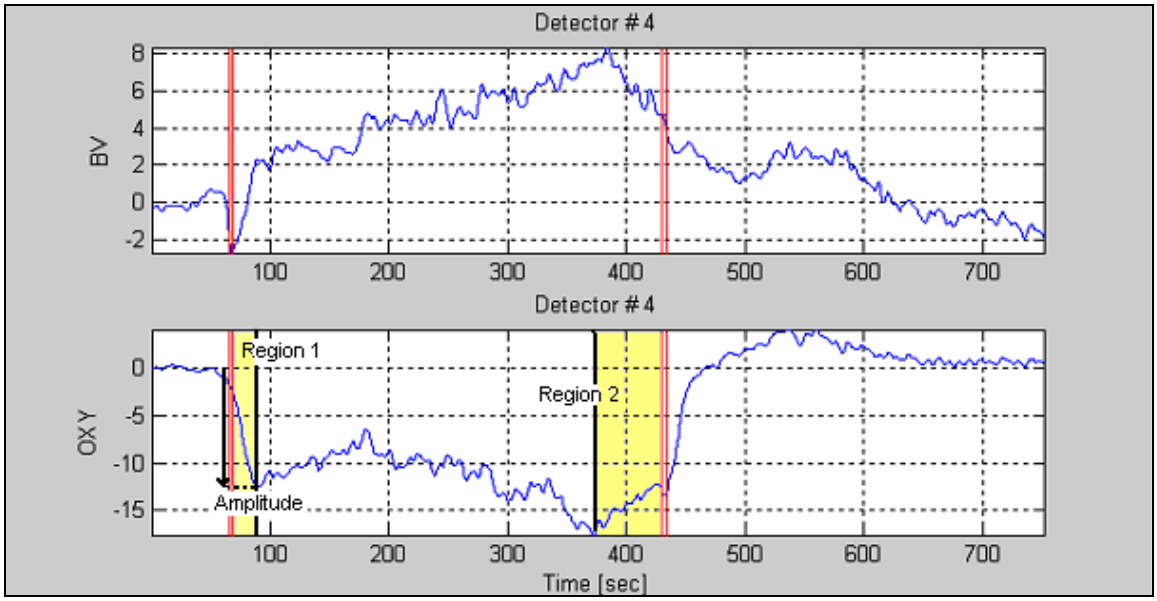

Figure 7.2 A typical sample indicating OXY parameter from NIRS measurements belong to the same subject from Group I.

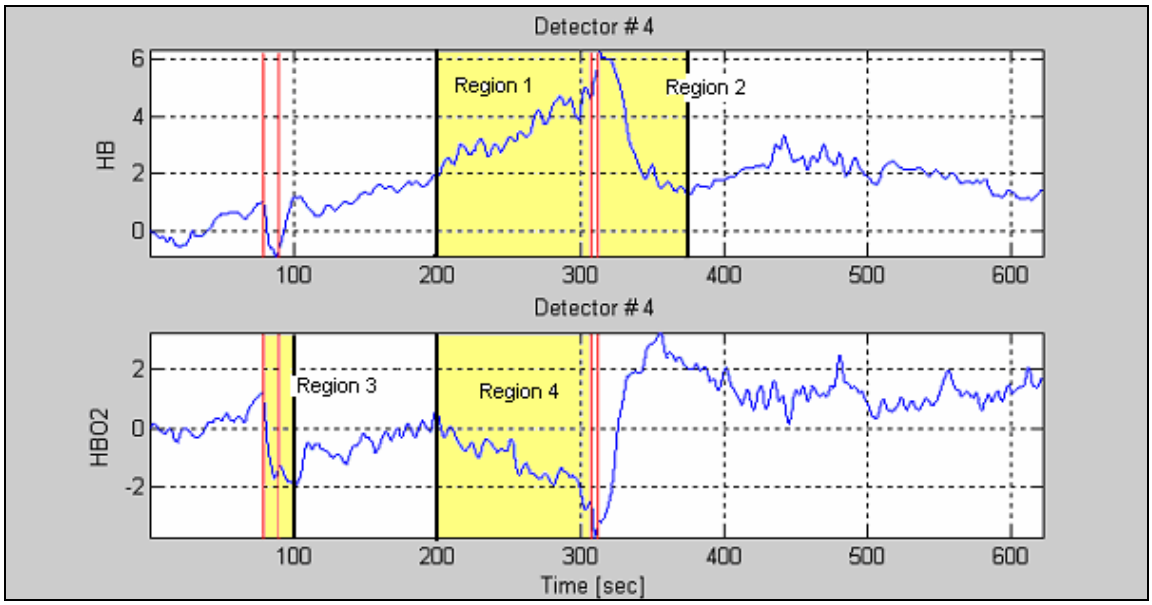

Figure 7.3 A typical sample indicating HB and HBO2 parameters belong to a subject from Group II.

Data from figure 7.3 represents HB and HBO2 signals measured from an irregularly exercising subject even if he is football player in school team, in group II. HB recovery slope and t1/2 parameters obtained from region two, are found to be 0,1083 and 21,4 seconds respectively which are relatively small values when compared to the subject from group I. For group II slope is expected to be smaller and t 1/2 is expected to be bigger when compared to group I due to deprivation of training induced alterations. In this

subject recovery slope is 0,1083 and t1/2 is 21,4 secods. Also, in the signal behaviour of $HBO_{2,}$ in region three, initial decline slope and amplitude were expected to be smaller which are determined as 0,1084 and 1,9 micromolar respectively.

Addionally, in region one and four, a clear drop in HBO2 and a steady increase in HB signal is seen which is supposed to be related to altered intramuscular pressure resulting in reduced O_2 tranfer as mentioned before.

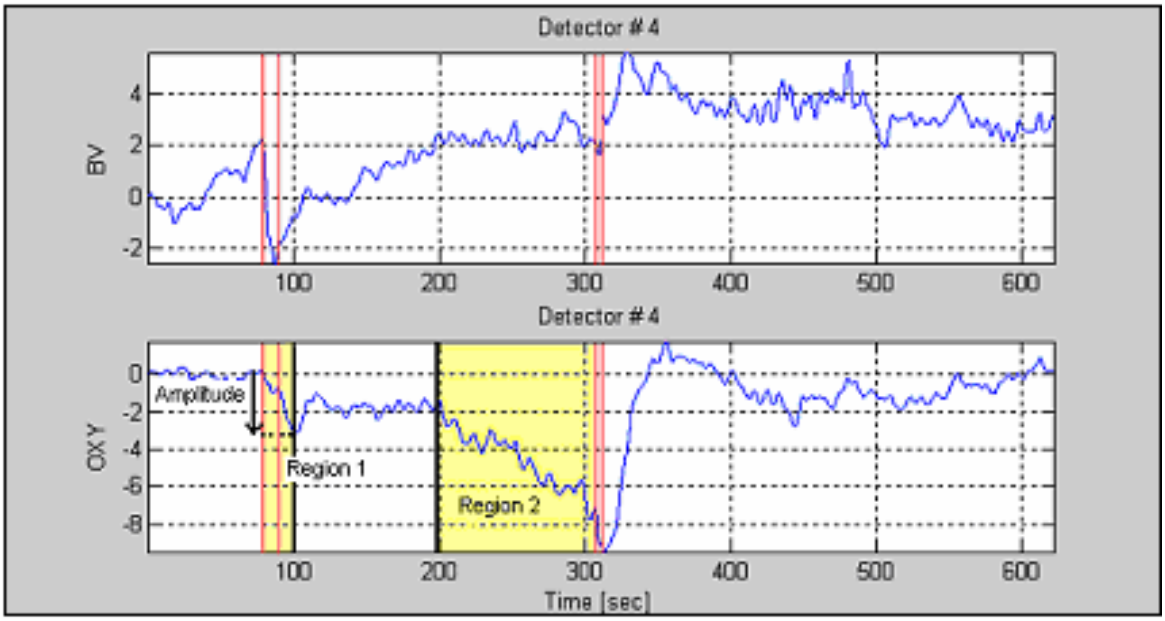

Figure 7.4 A typical sample indicating OXY parameter belong to the same subject from Group II.

Data from figure 7.4 suggests the same explanation about intramuscular pressure for region two, as well. In terms of OXY signal above data shows, initial decline slope and amplitude value, in region one, as 0, 3944 and 4,2 micromolar respectively and were expected to have the same behaviour in decline charactreristics with HBO2 parameters.

When these parameters compared, it is noticed that group II's subject has, %22 in HB recovery slope, %37 in recovery time, %39 in HBO2 decline slope, %43 in HBO2 decline amplitude, %20 in OXY decline slope and %60 in OXY decline amplitude lower values relative to group I's subject and exhibited %39 less performance in terms of squat exercise duration.

EMG parameters, as well, searched in terms of two groups, Group I and Group II according to squat exercise duration. MVC values are determined and EMG parameter slopes are examined in order to find similarities existing in each group. Table 7.1 and 7.2 shows percentage of MVC values for each group.

Table 7.1
Percentage of MVC (Maximum Voluntary Contraction) values of Group I.

Subject Initials	Total SQUAT (sec)	B.M.I	Max mV. MVIC	Max mV. Squad	%M.V.C. During Squat
K.S.	542	22,8	1,6075	0,5279	32,84%
A.K.	515	22,6	1,1444	0,3294	28,79%
Sa.K.	450	27,1	2,0954	0,4266	20,36%
M.E.	367	20,5	1,6486	0,4931	29,91%
U.A.	330	21,8	0,8051	0,2028	25,20%
Ö.Ç.	295	24,6	0,7575	0,2559	33,79%
Mean	416,5	23,2	1,3431	0,3726	0,28
Stdev	101,2	2,3	0,5293	0,1310	0,05

Table 7.2
Percentage of MVC (Maximum Voluntary Contraction) values of Group II.

Subject Initials	Total SQUAT (sec)	B.M.I	Max Mv. MVIC	Max Mv. Squad	%M.V.C. During Squat
M.A.	240	23,5	1,72314	0,31347	18,19%
L.T.	230	27,9	0,66857	0,27637	41,34%
M.P.	225	20,5	1,25268	0,34033	27,17%
Se.K.	220	25,1	0,94287	0,21106	22,38%
M.H.	210	22,6	1,05334	0,28820	27,36%
U.E.	190	21,7	0,74646	0,23278	31,19%
Mean	219,1	23,5	1,0645	0,2770	0,27
Stdev	17,4	2,6	0,3852	0,0485	0,07

As it is noticed, group I and II are almost matched in their BMI with the values of 23,2 ±2,3 and 23,5 ±2,6 respectively. Therefore, percentage of MVC of each group mached as well, 28 ±0,05 and 27 ±0,07 respectively, implying each group was exposed to nearly the same intramuscular pressure and occlusion due to exercise they performed

which was aimed at first. Figure 7.5, 7.6 and 7.7 depict the same subject's EMG data in Group I and figure 7.8, 7.9 and 7.10 depict the same subjects EMG data in Group II.

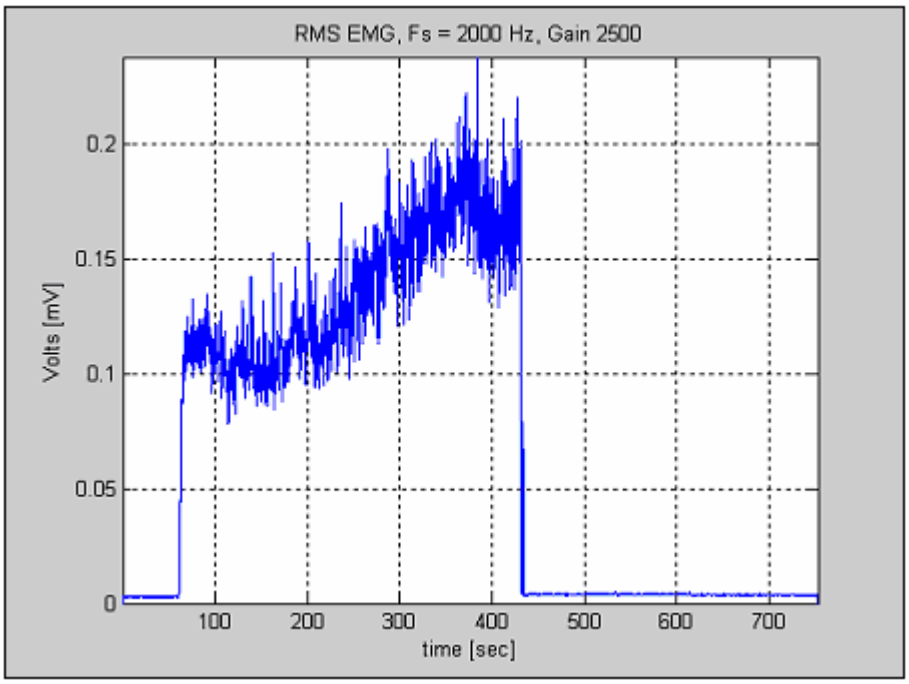

Figure 7.5 RMS of EMG measurement from the subject of Group I.

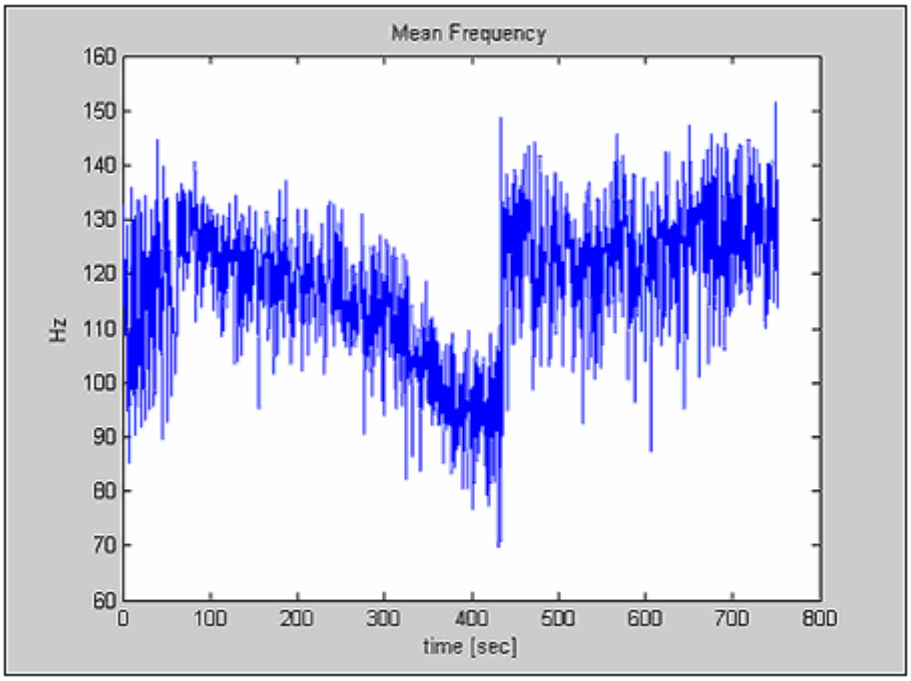

Figure 7.6 MNF of EMG measurement from the subject of Group I.

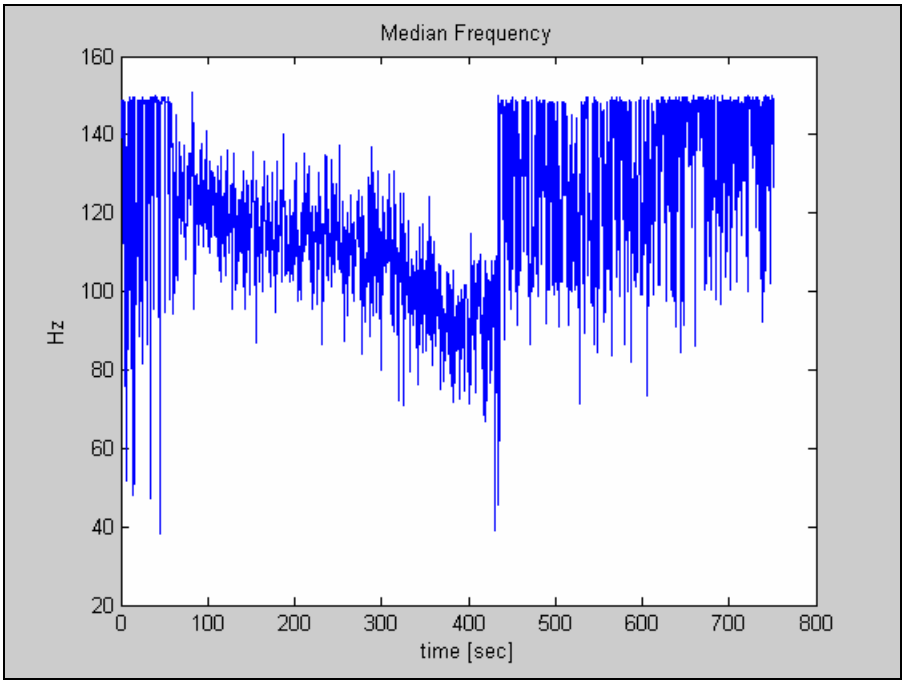

Figure 7.7 MDF of EMG measurement from the subject of Group I.

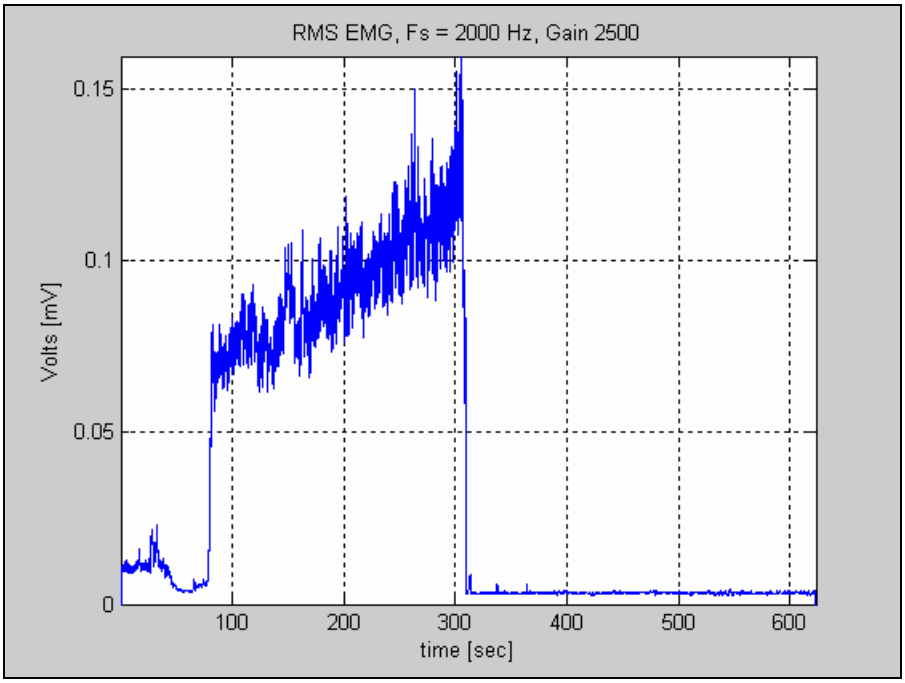

Figure 7.8 RMS of EMG measurement from the subject of Group II.

In group I's subject RMS, MNF and MDF slope values are determined as 0,00025, -0,09540 and 0,09522 respectively.

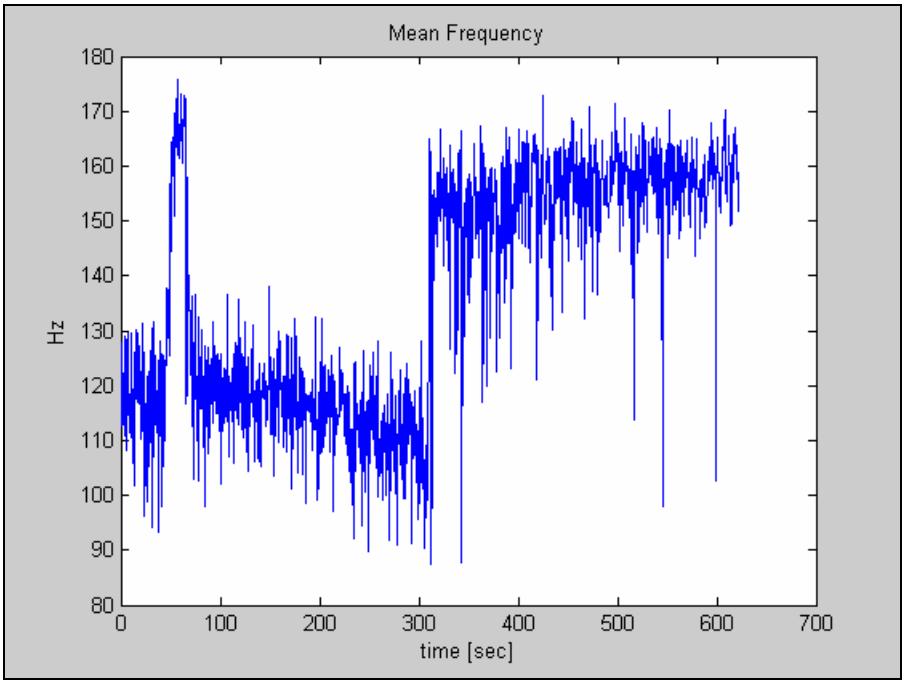

Figure 7.9 MNF of EMG measurement from the subject of Group II.

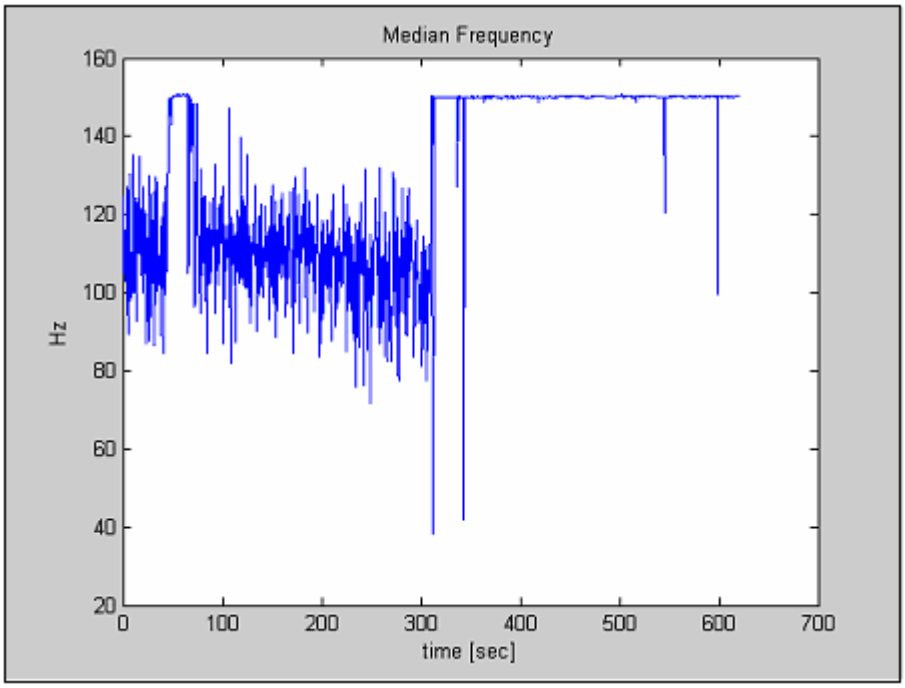

Figure 7.10 MDF of EMG measurement from the subject of Group II.

In group II's subject RMS, MNF and MDF slope values are determined as 0,00022, -0,04582 and -0,05141.

Knowing that the increase in EMG amplitude has been suggested to reflect fatigue induced changes in the exercising muscle [87] and RMS, MNF and MDF provide information about motor unit recruitment and/or firing rate, [36, 90, 92, 93, 94] changes in the muscle fiber propagation, muscle temperature, muscle fatigue and muscle fiber type, slopes of RMS, MNF and MDF are examined to understand whether the differences in mucle fiber type, motor unit recruitment, firing rates of motor units and muscle fiber propogation can be observed or not, in two groups.

It is seen that the subject belonging to group II has, %22 in RMS, %52 in MNF and %47 in MDF lower values relative to group I's subject .

Decrease MNF and MDF showed that conduction velocity clearly decreased with fatiguing squat exercise as explained in Sadoyama's study. Sadoyama et al. conducted constant load sustained isometric contraction exercises with different loads and suggested that such reductions may be due to the recruitment of new motor units with a slower conduction of action potential and/or the recruitment of active motor units with a faster conduction velocity, due to fatigue. They concluded that the changes in frequency parameters, MNF and MDF were related to changes seen simultaneously in the conduction velocity [88, 108, 109]. This finding suggests that in group I's subject recruitment of new motor units with a slower conduction of action potential and/or the recruitment of active motor units with a faster conduction velocity is seen more.

Also, Gabriel et al. examined whether an increase in synchronization of motorunit (MU) firing pattern occurs with training in normal subjects exerting medium to high levels of force as well as during sustained isometric contractions and noticed increased MU recruitment due to training [100] which poses an explanation for increased RMS, MNF and MDF slopes in group I's subject

Similar to our study Hagberg investigated the endurance time for sustained isometric exercise and dynamic exercise at different contraction levels. According to the report from his study; the rapid decrease in the endurance time at different sustained isometric contraction levels, for contraction levels above %15-20 MVC, has been attributed to an impairment of the blood flow, producing an ischemic effect due to the

increased intramuscular pressure [110]. Therefore, an accumulation of metabolites and a depletion of energy compounds occur within the muscle, impairing the contraction process and causing a localized mucle fatigue. Yet, in our study contraction levels were nearly the same so as the effect of occlusion.

However, recruitment of more MU's may be attributed to altered physical condition, recruitment of MU's with high firing rates indicates partially the use of type II fibers relatively. Additionally, EMG measurements are sensitive to temperature changes and temperature was not a controlled variable in this study. Findings of all parameters from fNIRS measurements acquired from 12 subjects resulted in these findings depicted in Table 7.3 and 7.4.

Table 7.3
Results of fNIRS measurements of Group I

Subject Initials	HB		HBO2 Decline Slope	Amplitude	OXY Decline Slope	Amplitude	Total Squat (sec.)
	Recovery Slope	$t_{1/2}$					
S.K.	0,2602	31,1	0,1076	3,6	0,2363	7,5	542
A.K.	0,1439	39,4	0,0919	2,5	0,2818	8,2	515
Sa.K.	0,0738	35,7	0,0654	2,7	0,0828	7,3	450
M.E.	0,1377	33,8	0,1775	3,3	0,4880	10,5	367
U.A.	0,0625	35,2	0,1255	2,7	0,3073	5,1	330
Ö.Ç.	0,0542	25,8	0,1020	1,8	0,1296	6,5	300
Mean	0,1130	33,5	0,1116	2,8	0,2543	7,5	417,3
Stdev	0,0786	4,6	0,0378	0,61	0,14385	1,8	100,0

Table 7.4
Results of fNIRS measurements of Group II

Subject Initials	HB		HBO_2 Decline Slope	Amplitude	OXY Decline Slope	Amplitude	Total Squat (sec.)
	Recovery Slope	$t_{1/2}$					
M.A.	0,0646	63,1	0,0471	1,3	0,1415	3,6	240
L.T.	0,0167	72,6	0,1004	1,7	0,0927	2,3	230
M.P.	0,1083	21,4	0,1084	1,9	0,3944	4,2	225
Se.K.	0,0510	37,3	0,1627	1,5	0,1120	0,9	220
M.H.	0,0597	36,4	0,0517	0,5	0,0798	2,4	210
U.E.	0,0418	48,3	0,1572	3,0	0,1556	5,9	190
Mean	0,0471	46,5	0,1046	1,3	0,1627	3,2	219,1
Stdev	0,0338	18,8	0,0495	0,8	0,1170	1,7	17,4

Data from table 7.3 and 7.4 are investigated statistically with a significance level (α) of 0.05. The result of t-test indicates that, difference between group I and group II is significant for HBO2 amplitude in 0.025, for OXY amplitude in 0.002 and for squat duration in 0.001 significance levels. However, other parameters have no difference between two groups. Also, the correlation is researched for all parameters in each group. However, no correlation is found between squat times and other parameters in group I and II.

Additionally, findings of all parameters from EMG measurements acquired from 12 subjects are presented in Table 7.5 and Table 7.6.

Table 7.5
Results of EMG measurements of Group I.

Subject Initials	MNF	MDF	RMS	Total Squat (sec)
K.S.	-0,00393	-0,01151	0,00024	542
A.K.	-0,03173	-0,02369	0,00020	515
Sa.K.	-0,00003	0,00170	0,00016	450
M.E.	-0,09540	-0,09522	0,00025	367
U.A.	-0,03456	-0,04253	0,00008	330
Ö.Ç.	-0,03105	-0,03602	0,00011	300
Mean	-0,03278	-0,03454	0,00017	417,3
Stdev	0,03416	0,03379	0,00007	100

Table 7.6
Resuls of EMG measurements of Group II.

Subject Initials	MNF	MDF	RMS	Total Squat (sec.)
M.A.	-0,00092	0,00005	0,00038	240
L.T.	-0,03850	-0,02627	0,00011	230
M.P.	-0,04582	-0,05141	0,00022	225
Se.K.	-0,09745	-0,05575	0,00027	220
M.H.	-0,04341	-0,03226	0,00314	210
U.E.	-0,11315	-0,13824	0,00030	190
Mean	-0,05654	-0,05064	0,00073	219,1
Stdev	0,04143	0,04733	0,00117	17,4

Datum from table 7.5 and 7.6 are investigated statistically with a significance level (α) of 0.05. The result of t-test shows that there is no significant difference between group I and group II for MNF, MDF and RMS parameters. Also, the correlation is researched for all parameters in each group and it is found that there is no correlation between the EMG parameters and squat duration. Additionally, in three subjects, one fom group I and the others from group II, MDF shift to lower frequencies is not seen. For that reason, to compare the two groups according to these parameters' slopes may not be a valid method in order to understand similarities in each group in terms of MU recruitment and firing rates. However, MDF shift to lower frequencies and icrease in RMS amplitude are seen in all subjects providing information about existence of fatigue.

Table 7.7 shows the statistical analysis of parameters gathered during squat exercise both from fNIRS and EMG measurements.

Table 7.7
Statistical Findings of the study

Instrument	PARAMETERS	GROUP I		GROUP II		P
		Mean	Stdev	Mean	Stdev	
NIRS	**HB Rec. Slp.**	0,113	0,0786	0,0471	0,0338	0,085
	HB Rec. t1/2	33,5	4,6	46,5	18,8	0.132
	HBO2 Dec.Slp.	0,1116	0,0338	0,1046	0,0495	0,787
	HBO2 Dec. Ampl.	2,8	0,61	1,3	0,8	**0,025***
	OXY Dec. Slp.	0,2543	0,1438	0,1627	0,117	0,254
	OXY Dec. Ampl.	7,5	1,8	3,2	1,7	**0,002***
EMG	**RMS Slp.**	0,00017	0,00007	0,00073	0,00117	0,269
	MNF Slp.	-0,03278	0,03416	-0,05654	0,04143	0,304
	MDF Slp.	-0,03454	0,03379	-0,05064	0,04733	0,513
Subject Squat Duration		416,5	102,2	219,1	17,4	**0,001***

Apart from these findings, when mean values are considered, in the less exercising group it is seen that HB recovery slope is %59, HBO2 decline slope is %77, HBO2 amplitude is %54, OXY decline slope is %37 and OXY apmlitude is %58 small and recovery time is %38 big relative to exercising group.

8. DISCUSSION

The major findings of this study demonstrate that all parameters of NIRS measurements may point out higher O_2 exctraction capability of muscle in Group I which may be releted to elevated endurance due to higher exercising frequency. However, one handicap in our study, which probably have an influence on our findings especially on the statistical data, was the group specification in their exercise frequency. The subjects who paticipated in this study were neither totally athlete nor sedantary. What is common for all the subjects is that there are decreases at beginning of exercise in HBO_2 and OXY signals indicating O_2 extraction from the tissue then continuing with an increase possibly due to compensation mechanisms of the muscle such as cardiovascular response and dilation of capillaries.

Consistent with the findings of this study Kalliokoski et al. [23] investigated differences in oxygen uptake and blood flow in skeletal muscle between endurance-trained and untrained subjects and found that oxygen extraction fraction was significantly higher in the exercising muscle in athletes. In addition blood transit time in the exercising muscle was longer and perfusion was more homogeneous, which may be associated with enhanced oxygen supply to muscle cells, resulting in improved exercise efficiency in the exercising muscle in the trained subjects. Additionally it is known that training induces adaptive changes in skeletal muscle, such as increases in oxidative fiber types (type I) which possess a higher capillary density and oxidative potential than type II fibres [22, 111].

In another study, Costes et al. examined the effects of endurance training on muscle oxygen saturation measured by NIRS in a group of healthy untrained subjects and demonstrated that NIRS was sensitive to changes in intracellular metabolism. They found that muscle deoxygenation was significantly reduced posttraining [65].

Additionally, Neary et al. suggested that muscle oxygenation measured by NIRS reflects the balance between O_2 supply and consumption in skeletal tissue and hypothesized that, with the type of endurance training performed in their study, level of

muscle deoxygenation was related to a number of peripheral factors, including an increased capillarization, elevated mitochondrial density, and enhanced activity of oxidative enzymes, all of which most likely augmented the arterio venous O_2 difference as a result of training [66].

One impressing point from NIRS data in this study is HB signal characteristics when recovery phase is concerned implying lactate removal and enhanced capillary density. It has been established that muscle oxidative capacity has been significantly improved by endurance training [112, 113, 114] and oxidation is the major metabolic pathway for lactate disposal during rest, sustained and sub-maximal exercises, and recovery [114, 115, 116, 117] and it is also known that an individual's maximal sustainable power output or speed is highly related to their lactale threshold [118, 119].

In addition to this, Hawley reported that, endurance training is associated with an increase in the activities of key enzymes of the mitochondrial electron transport chain and a concomitant increase in mitochondrial protein concentration. These morphological changes, along with increased capillary supply, result in a shift in trained muscle to a greater reliance on fat as a fuel with a concomitant reduction in glycolytic flux and tighter control of acid-base status. Taken collectively, these adaptations result in an enhanced performance capacity [119].

Thomas et al. [114] proved that training status influenced the relationship between blood lactate removal ability and the maximal muscle oxidative capacity associated with the delay in the fatigue. In relation to this particular study, in terms of HB recovery times, it is seen that more exercising group obviously had the adaptations, which increase with training such as the rise of lactate transporters in the sarcolemmal membrane [113] enhanced capillary density [120] and enhanced mitochondrial content.

Also, Stanley et al. [121] showed that the working skeletal muscle not only is a major site of blood lactate removal during exercise, but also appears to consume lactate during recovery [115, 116]. Moreover, faster removal during recovery in the well-trained subjects compared with the untrained or little-trained subjects could be explained by an

increase in mitochondrial volume and density induced by training. In this study, faster recovery of HB in group I than group II can be explained by above suggestions.

Strikingly some authors [122, 123, 124] have reported a negative correlation between fast twitch fibers and knee extension isometric endurance time implying that isometric endurance may also be influenced by the fiber composition of a muscle which is consistent with endurance times of our study. lt was suggested that shorter endurance times were a result of increased lactate production by FT fibers [122, 123] which may be reduced to some extent and result in enhanced oxydative capacity in trained individuals.

Several studies showed that effect of temperature is another factor affecting muscle performance. Elevation of deep muscle temperature of only a few degrees centigrade has been shown to reduce isometric endurance significantly [125]. Edwards et al. found that passive heating of a working muscle resulted in an increase in muscle lactate levels. It was hypothesized that the accumulation of hydrogen ions would inhibit ATP regeneration and induce a quicker onset of fatigue during isometric endurance exercise [126]. In animal studies it has been shown that fatigability of fast twitch and slow-twitch fibers was affected by changes in deep muscle temperature [128], and in human studies the rate of heat production during an isometric contraction was found to correlate highly with FT fiber percent [127, 129]. However**,** in our study, room temperature and exercising muscle temperature was not measured.

It has been accepted that the marked reduction in force output during isometric contractions results from ischaemia due to e1evated intramuscular pressure or increasing concentrations of metabolites are associated with fatigue, such as H+, K+, Pi [108] or NH3 [130]. During sustained isometric contraction, the fatigue threshold corresponds with limitations in blood flow [131] and excitation contraction coupling failure, or impairment of cross-bridge cycling in the presence of unchanged or increased neural drive [132, 133].

To understand fatigue mechanism Duchateau and Hainaut suggested that two different processes are involved during muscle fatigue in their 60 sec. sustained isometric forearm experiment -coherently with that of Karlsson [134]- during contraction, anaerobic alactic metabolism plays the major role for about 20 s, and thereafter anaerobic lactic

processes take over, later lactic acid and H^+ are produced and intracellular pH is consequently reduced, leading inhibition of interaction among contractile proteins [135, 136]. As a result, fewer cross bridges are formed and tetanic tension is now smaller and its time development is slower, since fewer contractile units contribute to stretch passive elastic elements [137]. The observed slower relaxation in fatigued muscle is also coherent with the hypothesis of reduced pH. Low pH was indeed shown to maintain increased cytosolic Ca^{2+}concentration [138] and suggested to inhibit cross-bridge dissociation, possibly by "a direct effect on Ca^{2+} uptake by sarcoplasmic reticulum or a combined effect with other factors, such as ADP accumulation [139] rather than ATP depletion [110]. It is conluded that intracellular processes play the major role in contractile failure during sustained and intermittent contractions [140].

Although in our research it could not be possible to reveal MU behaviour with current investigational method. It is known that the behaviour of motor units (MUs) change and recruitment of additional MUs is seen during sustained contractions due to muscle fatigue development and decline of firing rate has been documented [141]. De Luca et al. found that the rate of decrease in firing rate was found to have a systematic trend related to the recruitment threshold and average firing rate of the motor unit. Specifically, motor units with lower recruitment thresholds and greater average firing rates decreased their firing rates more slowly than motor units with higher recruitment thresholds and lower average firing rates [142].

Additionally, in Hagberg's study [110] a nonlinear relationship found between RMS amplitude and time during the fatiguing exercises which have also been reported by other investigators. Kuroda et al. [143] reported an exponential increase of integrated EMG from the rectus femoris during a fatiguing isometric contraction. Development of muscle fatigue was well correlated with the changes of both the myoelectric amplitude and the MDF. The time constants of the RMS myoelectric amplitude and the MDF were correlated with both the contraction level and the endurance time in the studied exercises [96].

Also, the MNF decrease and the increase of amplitude in a fatiguing contraction mainly results from a conduction velocity decrease in the muscle fibers due to alteration in

the energy metabolism [108]. A decrease in the conduction velocity of the muscle fiber causes a shift in the power spectra toward lower frequencies [109]. The muscle tissue and the surface electrodes act as low-pass filters of the myoelectric signal [108]. Thus more signal energy passes through the tissue when a shift to lower frequencies occurs in the myoelectric power spectrum. This is recorded by surface electrodes as an increase of RMS amplitude [144].

Moritani [146] proposed a hypothesis that involves an attempt by the nervous system to generate muscle force while avoiding peripheral neuromuscular transmission failure. Continued high MU firing rates would eventually impair excitation and contraction coupling through either a depletion of Na^+ or ATP. Therefore, the nervous system decreases MU firing as a protective mechanism. Moritani cites the observation of a reduction in MU firing during sustained maximal isometric contractions before any evidence of neuromuscular transmission failure in support of his hypothesis. The increase in EMG amplitude and a decrease in MDF interpreted as an increase in MU synchronization [145]. MU snchronization appears sporadically in surface EMG recording as large periodic waveforms [145, 147] and it is produced by increased recruitment and firing frequency at the same time [95, 145]. The increased probability for temporal overlap then results in an increase in EMG amplitude as observed here [95, 145].

The second possible mechanism is based on Basmajian's theory of "progressive inhibition." This theory suggests that the nervous system, as it acquires a new motor skill, learns to minimize antagonist coactivation and extraneous muscle activity by means of training. In this view, optimal isometric strength is obtained when agonist muscle contractions occur without antagonist coactivation [149].

Carolan and Cafarelli found that biceps femoris EMG activity during knee extension decreased 20 per cent after eight weeks of isometric knee extension strength training whereas knee extension strength increased 32.5 per cent. The authors emphasized the importance of antagonist coactivation reduction by pointing out that this strength increase occurred without a rise in vastus lateralis EMG activity [148].

Consistent with above explanations in this study RMS increase MNF and MDF decrease are observed. This objective measurement enabled a prevention from scientific error which may be revaled by subjective inspection. Additonally, if increase in EMG amplitude is taken as an indicator of fatigue, it is seen that group I maintains the exercise for a longer time with the existance of increased EMG signal than group II which may be resulted from increased lactate treshold due to training or psychological factors.

9. CONCLUSION

The results of this study showed that HBO2 amplitude and OXY aplitude parameters can be used as good predictors for evaluation of muscular performance and endurance. Therefore, the use of NIRS can be beneficial in having information about muscular performance in terms of muscular endurance status by assessing levels of tissue deoxygenation trends in evaluating metabolic alterations in exercising muscle. Moreover, it is concluded that a longer duration of isometric contraction is related to an increased oxygenation status of the muscle and higher O_2 exctraction capability of muscle is related to muscular endurance.

The results of this study showed agreement with the widely accepted use of EMG as a beneficial tool in the assesment of muscular fatigue and detection of fatigue development. However, investigating solely the slopes of surface EMG parameters used in this study was found to be insufficient to evaluate muscular endurance and it is concluded that a more broad investigation of these parameters is required.

A limitation of the present study was the handicap in subject classification and inability to supply homgeneity in physical condition of the subjects of group 1 and group 2. Such handicap might have an important influence on our measurements and statistical results.

In addition to the insight derived from this study about muscle O_2 kinetics and influence of training on muscular endurance, investigations using similiar exercise protocols which should be performed with more controlled variable such as blood pressure, heart rate, gas exchange cardiac output, blood lactate level measurement can be beneficial.

APPENDIX A. fNIRS MEASUREMENTS

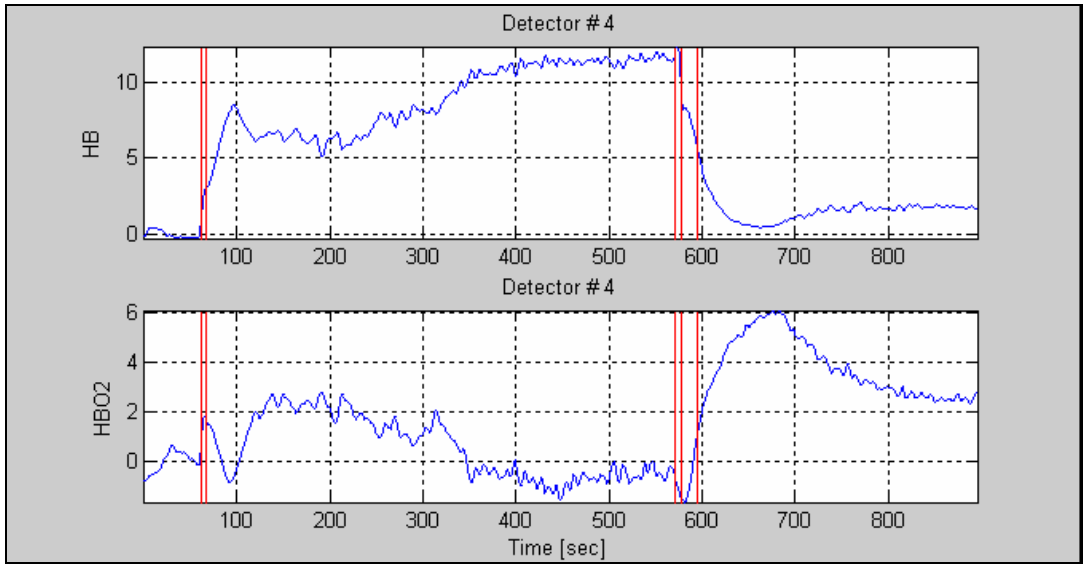

Figure A.1 Subject AK's HB and HBO_2 measurements.

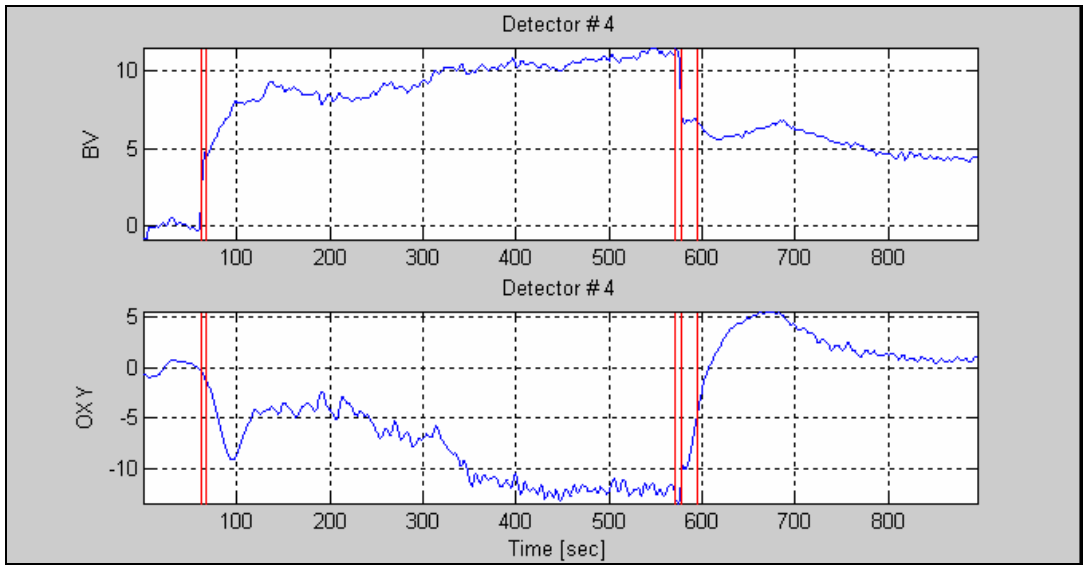

Figure A.2 Subject AK's BV and OXY measurements.

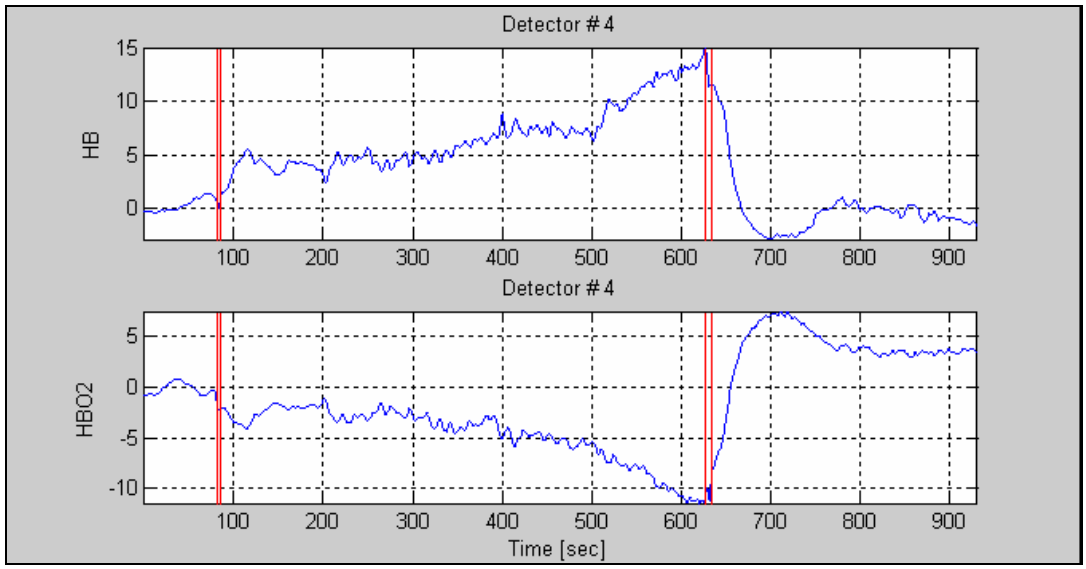

Figure A.3 Subject KS's HB and HBO_2 measurements.

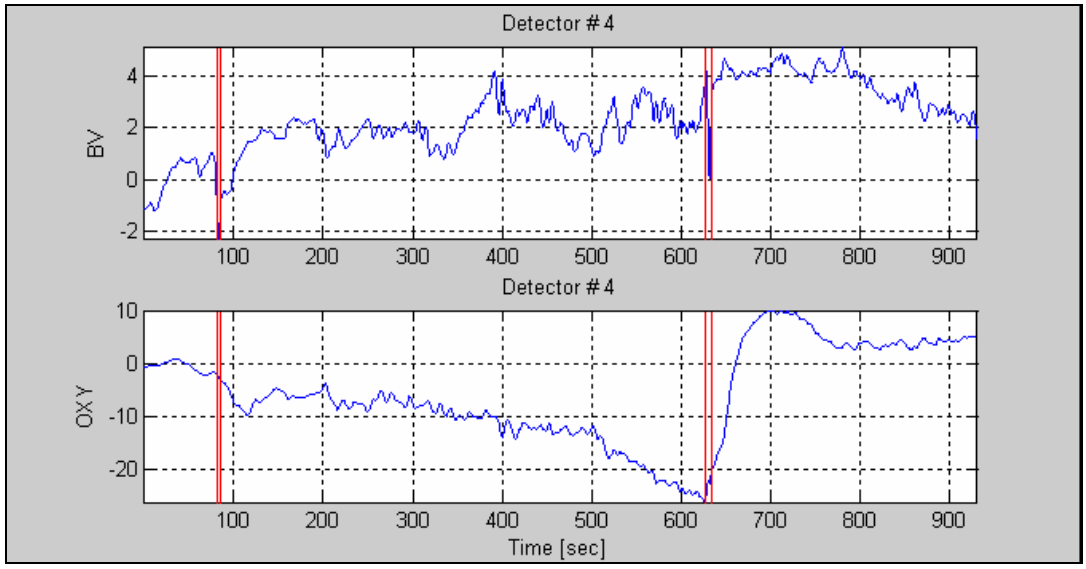

Figure A.4 Subject KS's BV and OXY measurements.

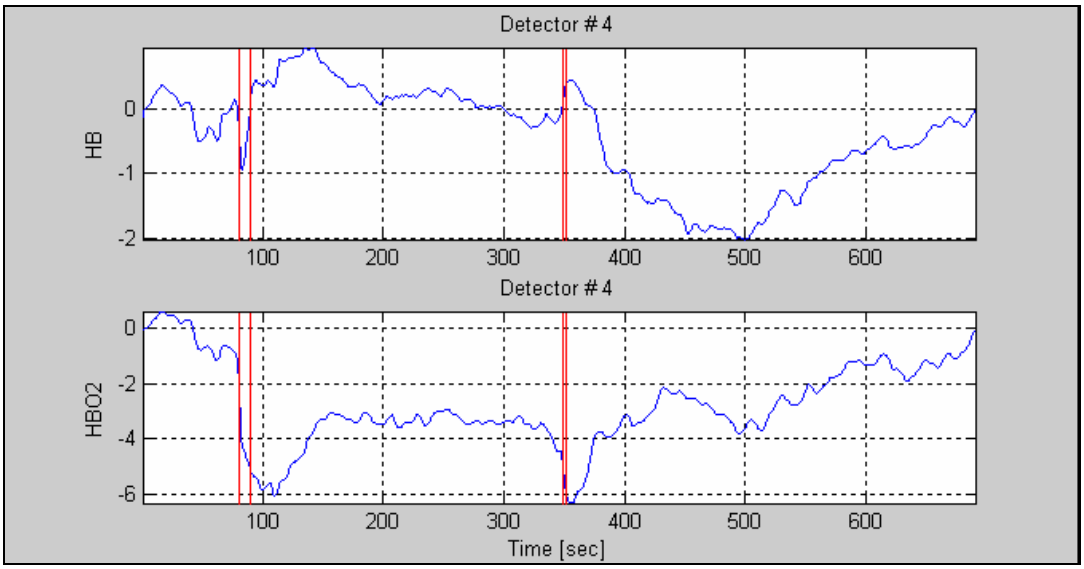

Figure A.5 Subject LT's HB and HBO_2 measurements.

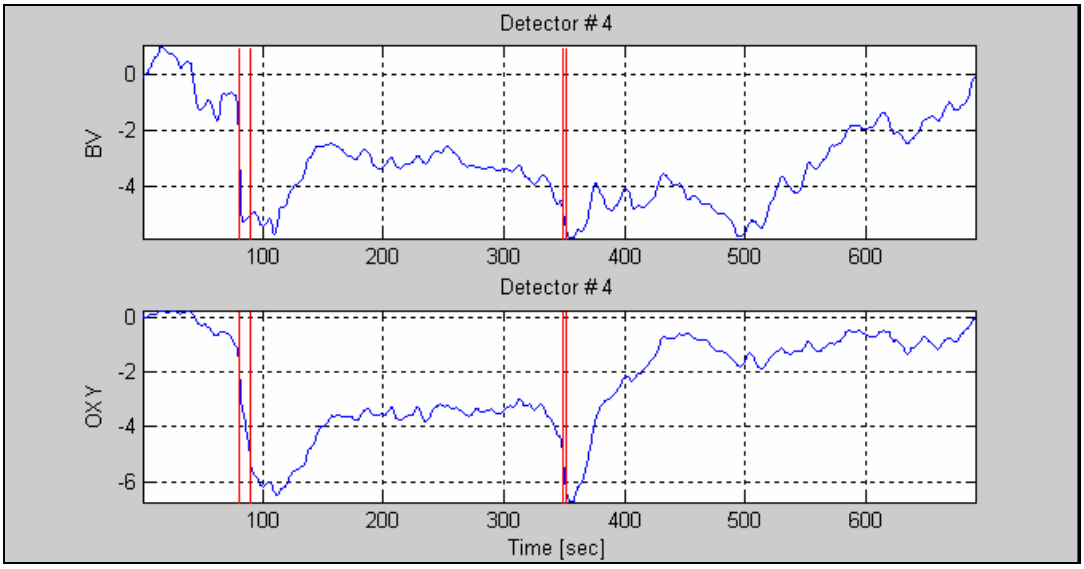

Figure A.6 Subject LT's BV and OXY measurements.

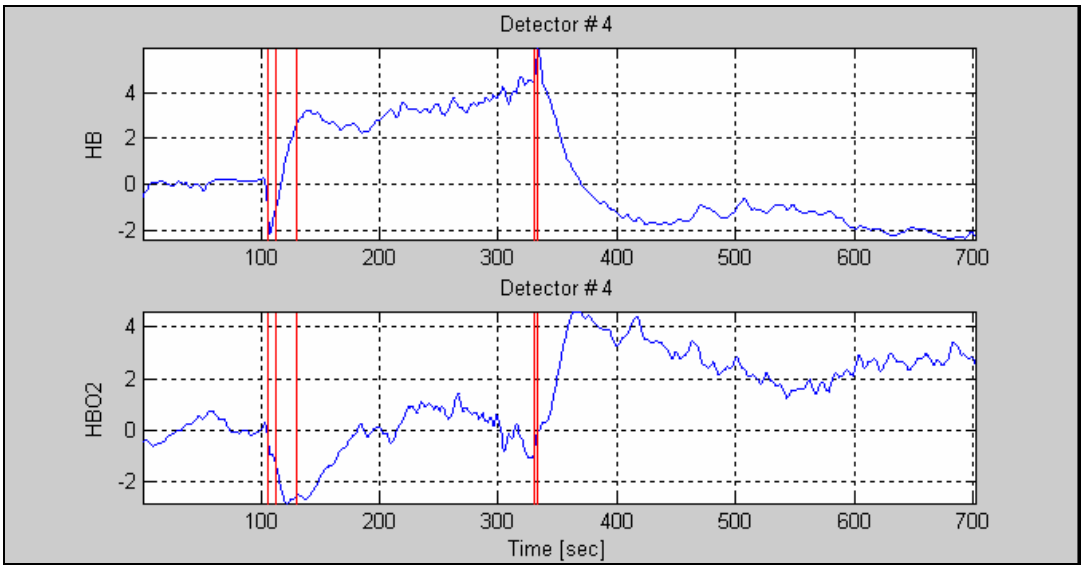

Figure A.7 Subject MH's HB and HBO_2 measurements.

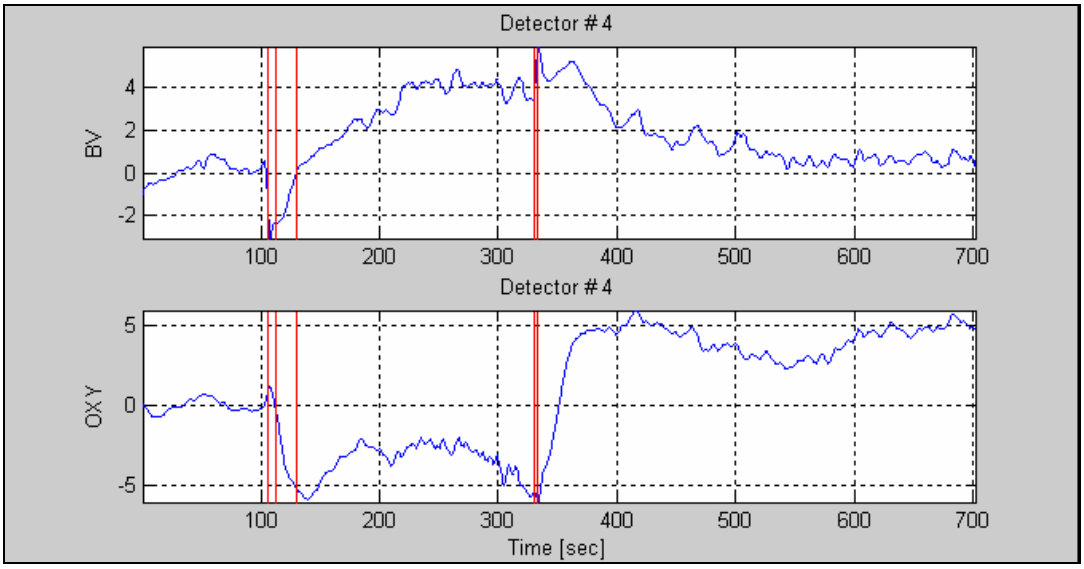

Figure A.8 Subject MH's BV and OXY measurements.

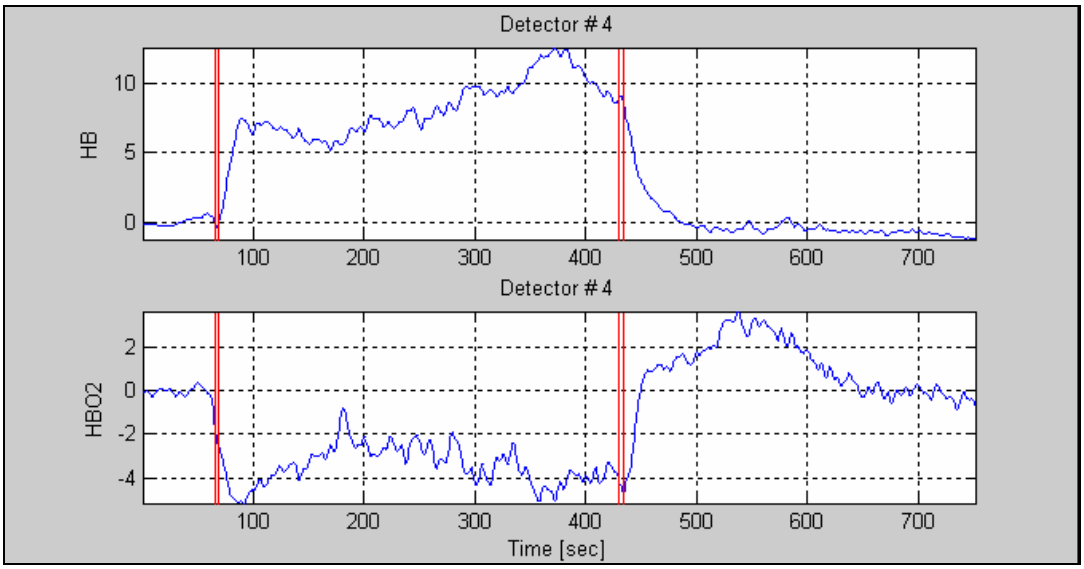

Figure A.9 Subject M.E's HB and HBO_2 measurements.

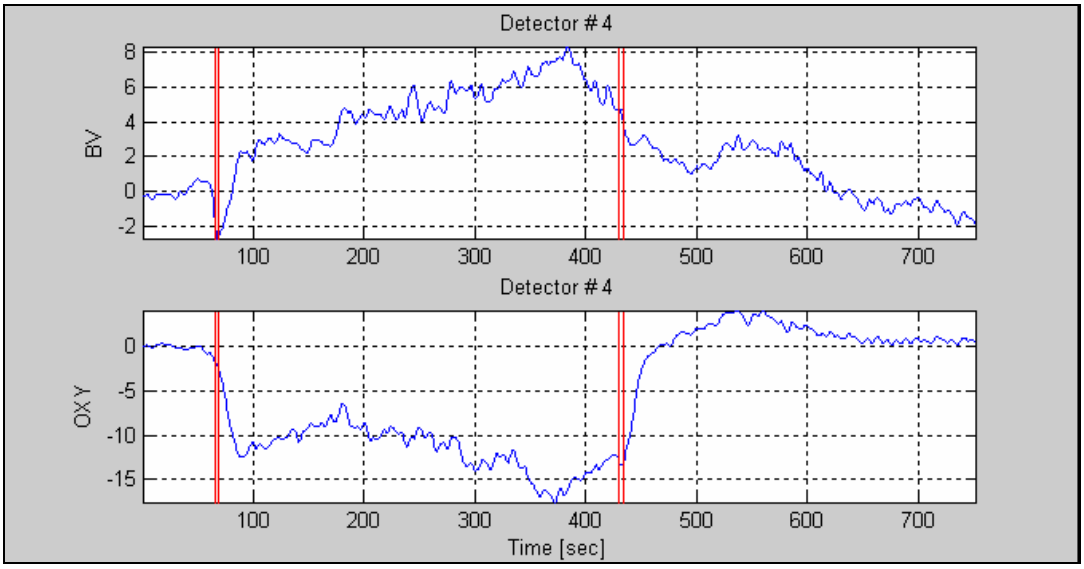

Figure A.10 Subject ME's BV and OXY measurements.

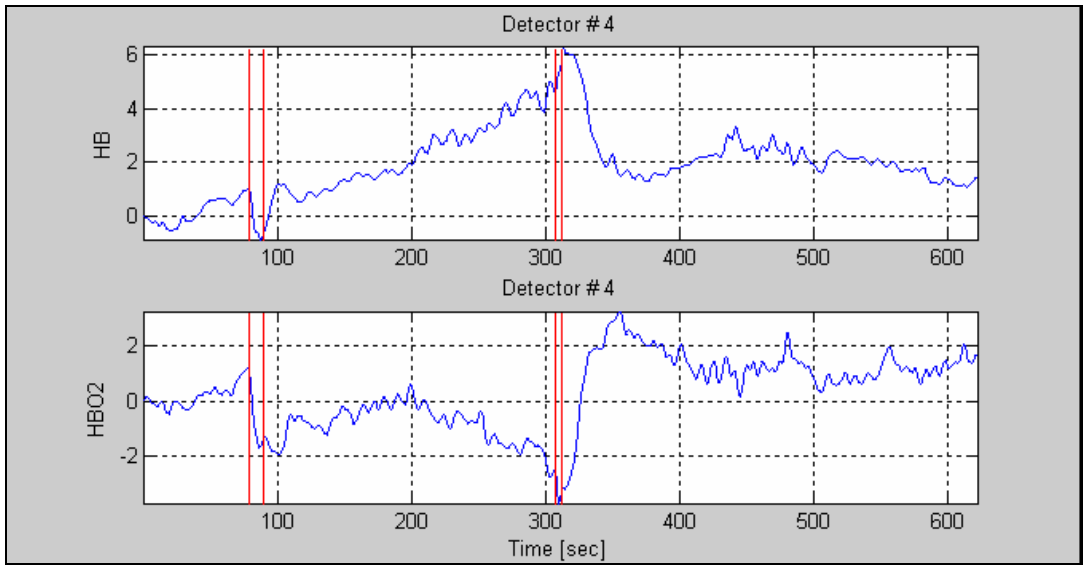

Figure A.11 Subject M.P's HB and HBO_2 measurements.

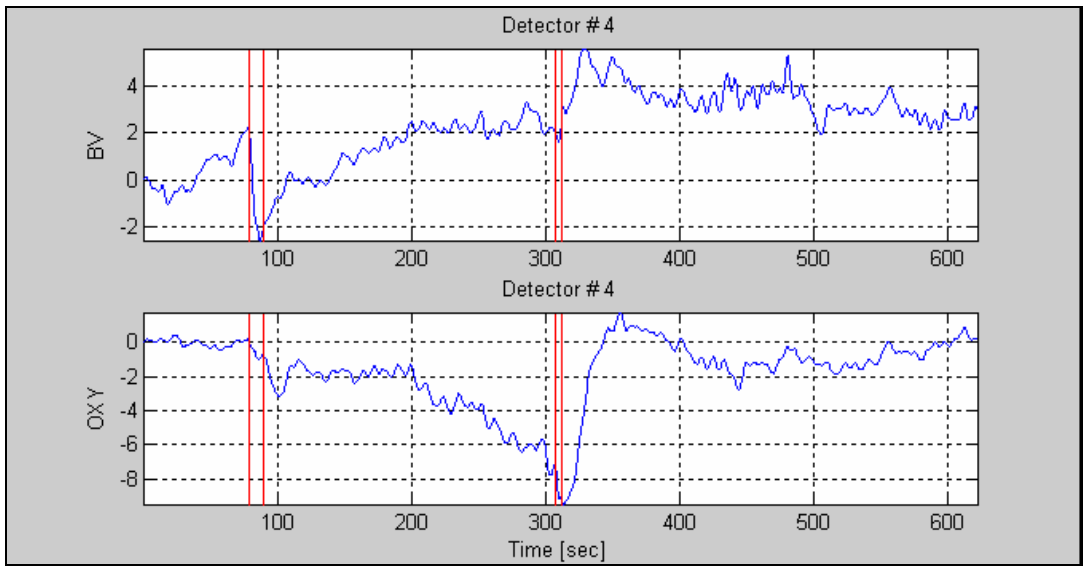

Figure A.12 Subject MP's BV and OXY measurements.

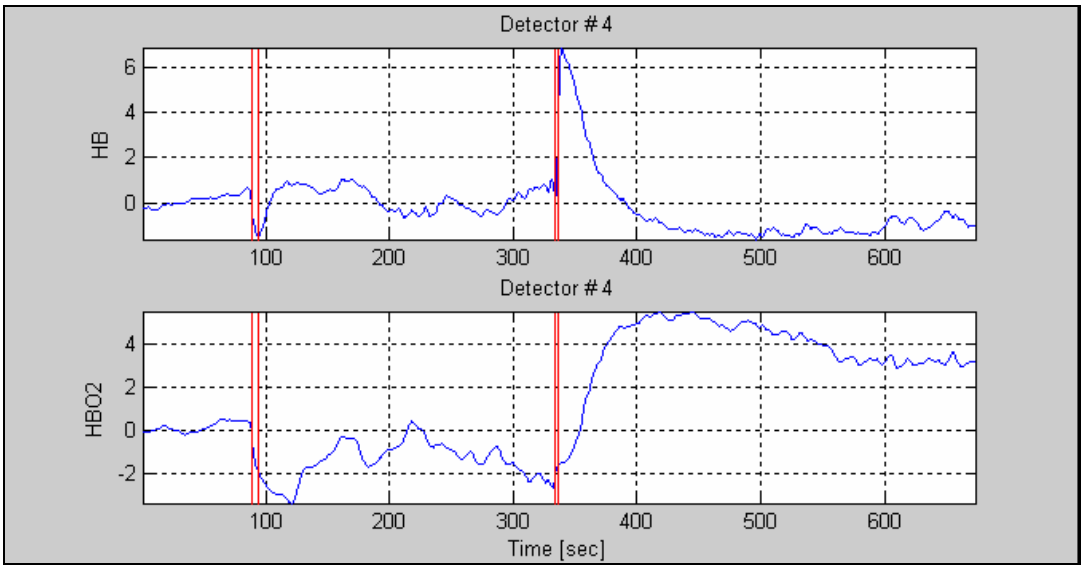

Figure A.13 Subject M.A.'s HB and HBO_2 measurements.

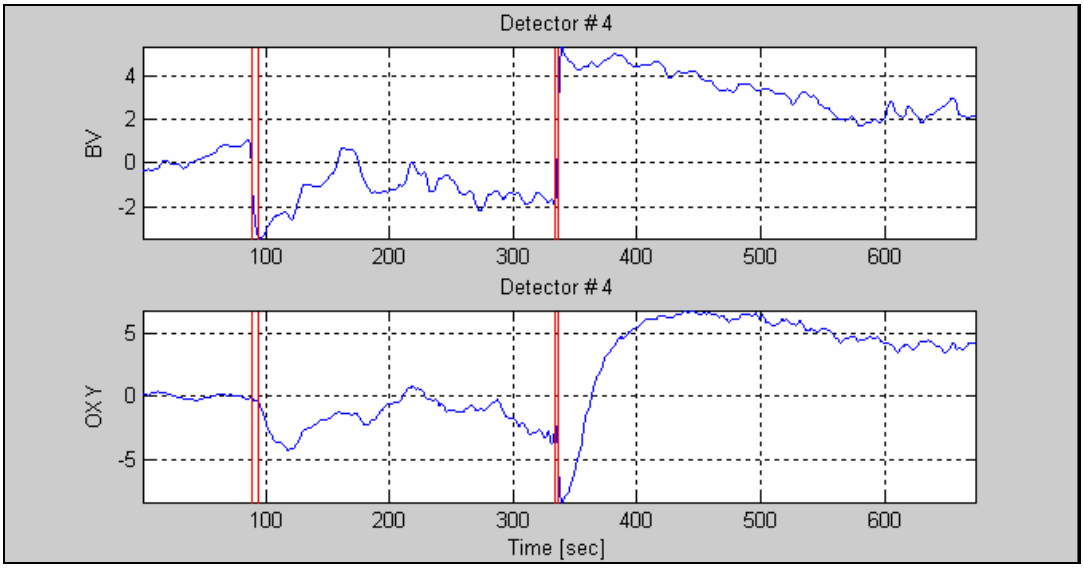

Figure A.14 Subject MA's BV and OXY measurements.

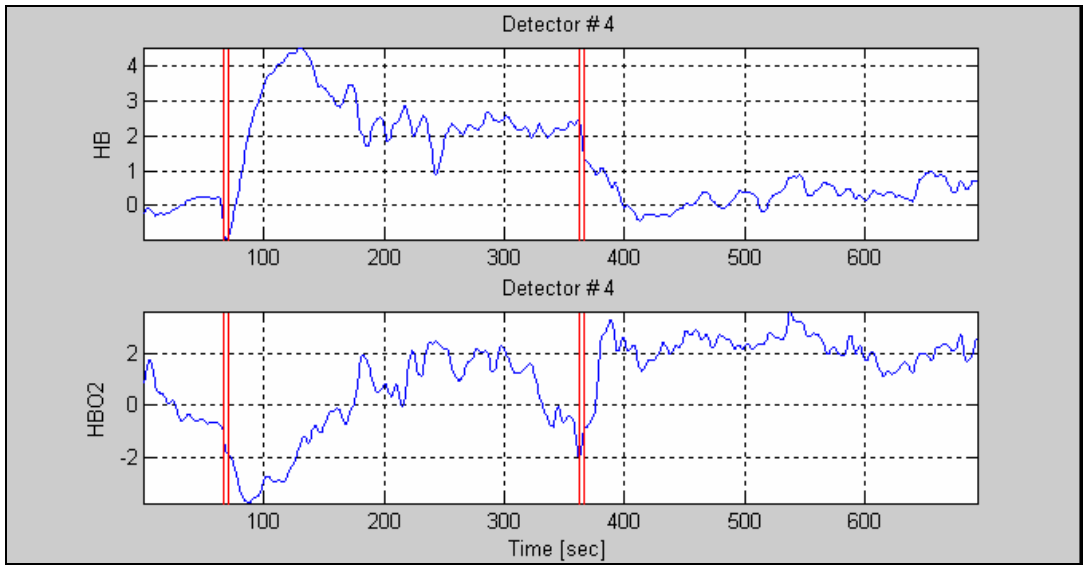

Figure A.15 Subject OC's HB and HBO_2 measurements.

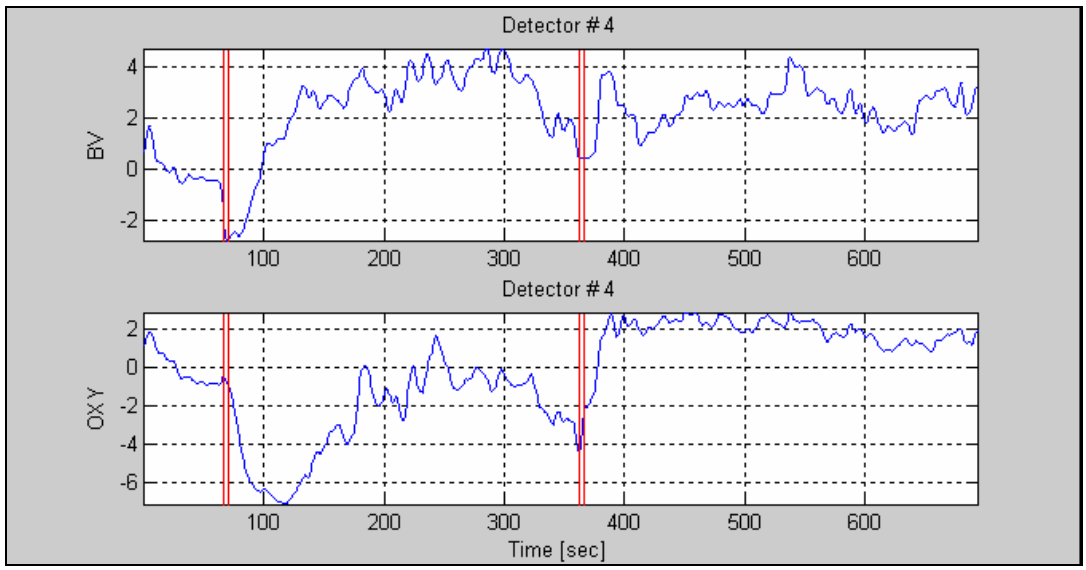

Figure A.16 Subject OC's BV and OXY measurements.

Figure A.17 Subject SaK.'s HB and HBO_2 measurements.

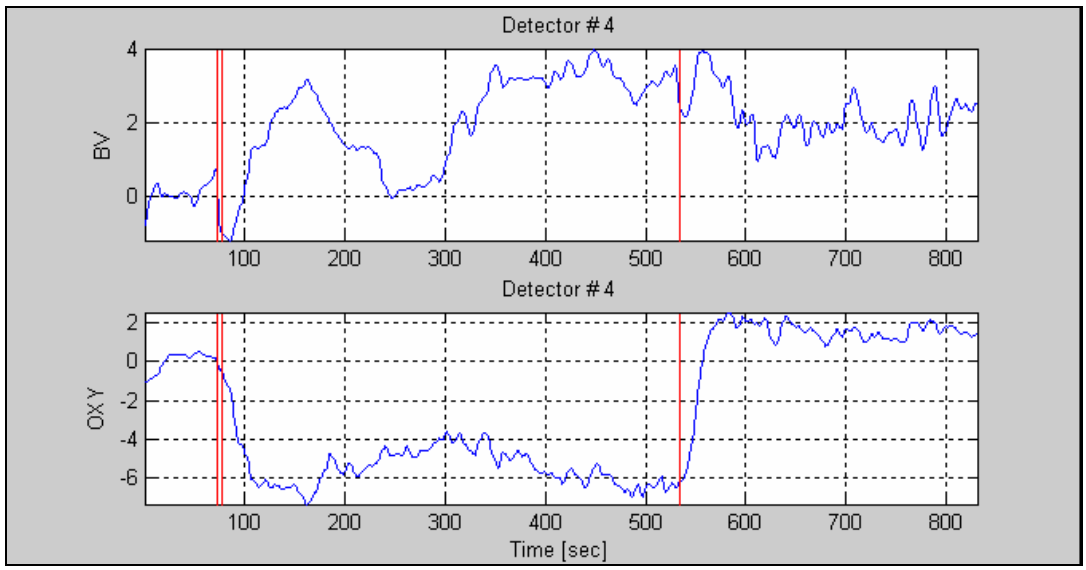

Figure A.18 Subject SaK's BV and OXY measurements.

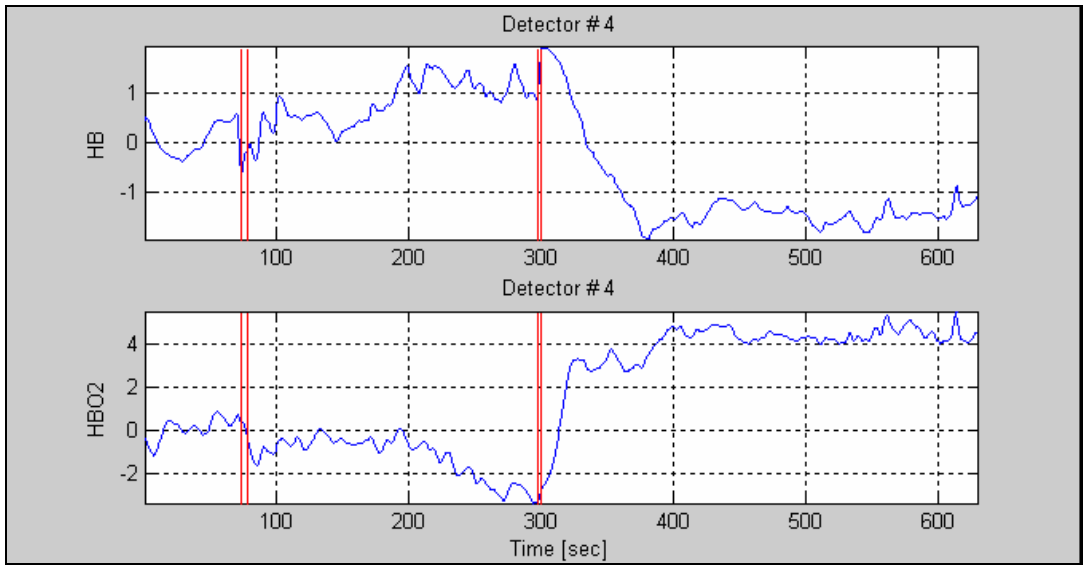

Figure A.19 Subject SeK.'s HB and HBO_2 measurements.

Figure A.20 Subject SeK's BV and OXY measurements.

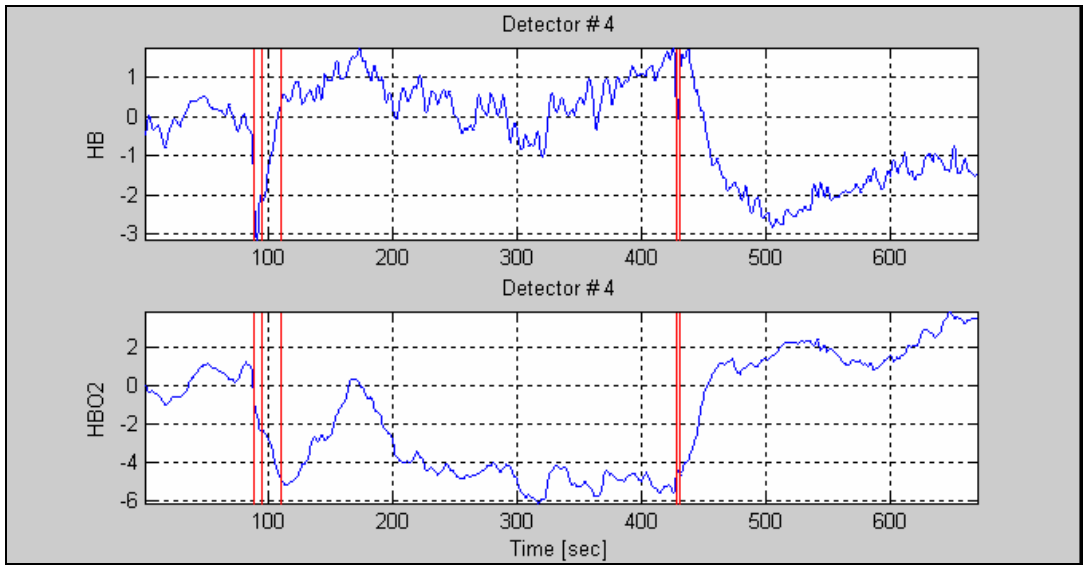

Figure A.21 Subject UA's HB and HBO_2 measurements.

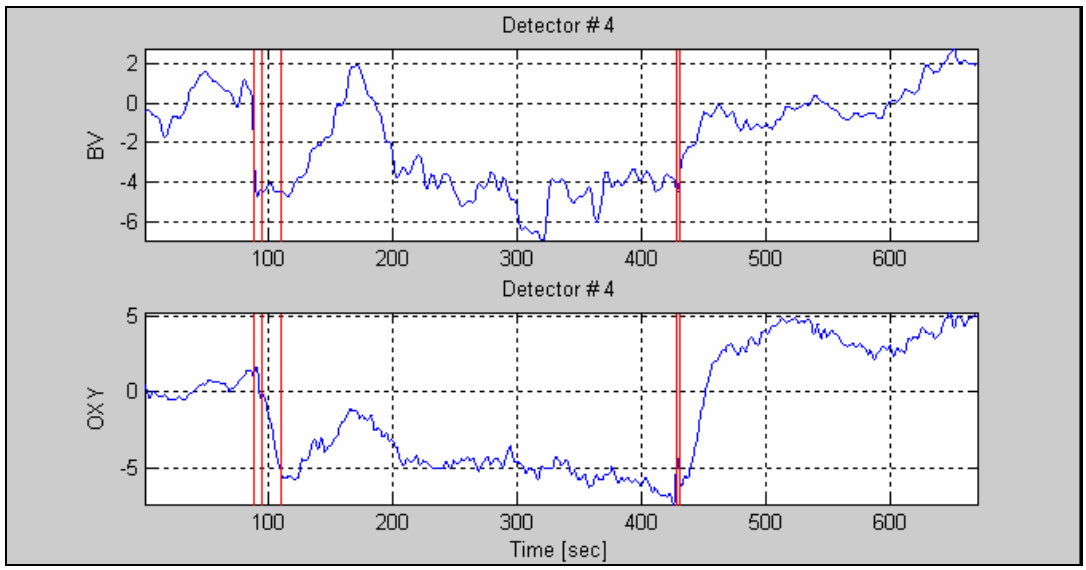

Figure A.22 Subject UA's BV and OXY measurements.

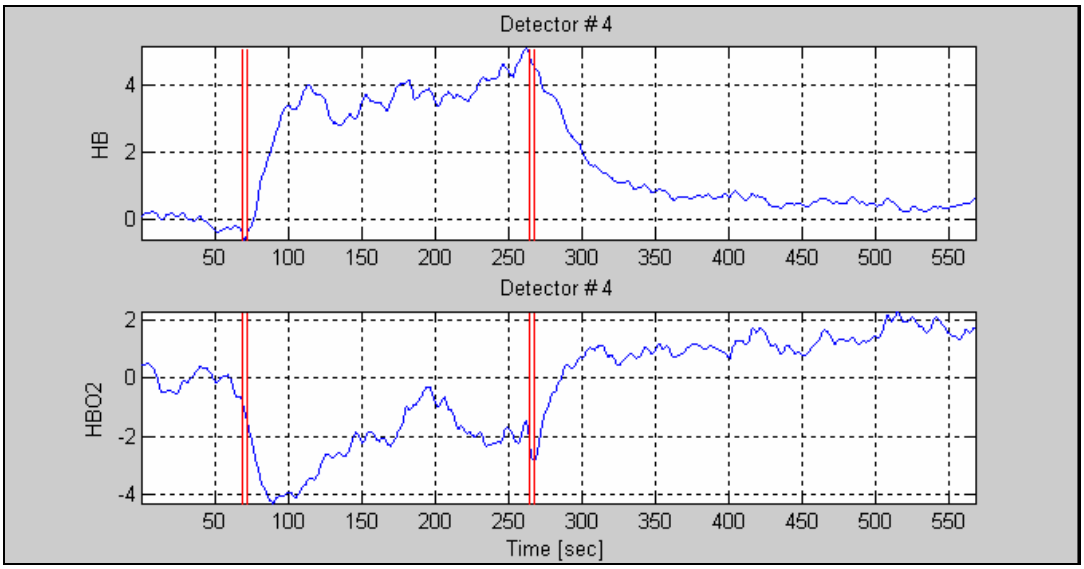

Figure A.23 Subject UE's HB and HBO_2 measurements.

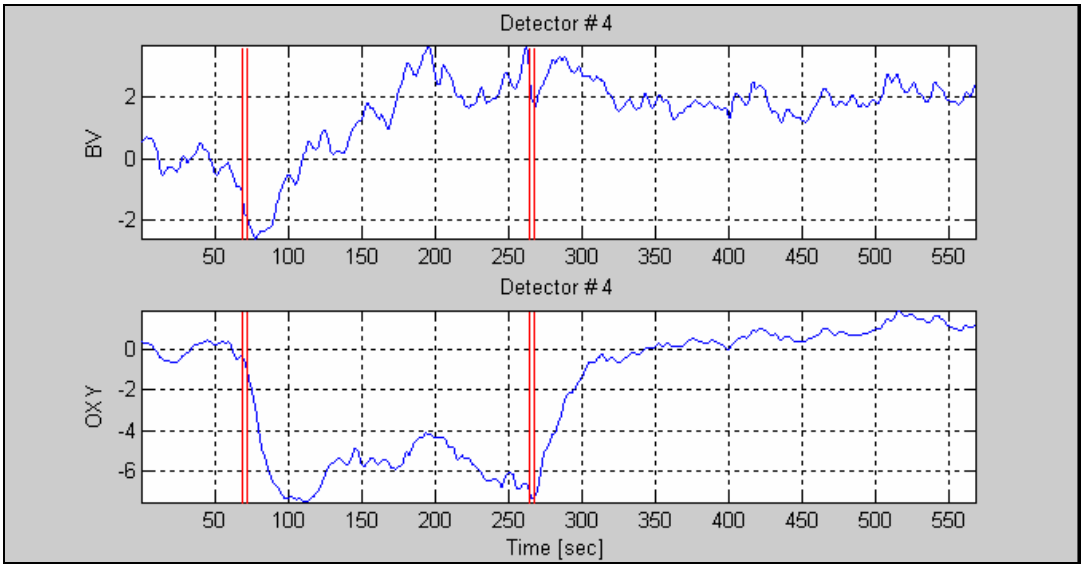

Figure A.24 Subject UE's BV and OXY measurements.

APPENDIX B. EMG MEASUREMENTS

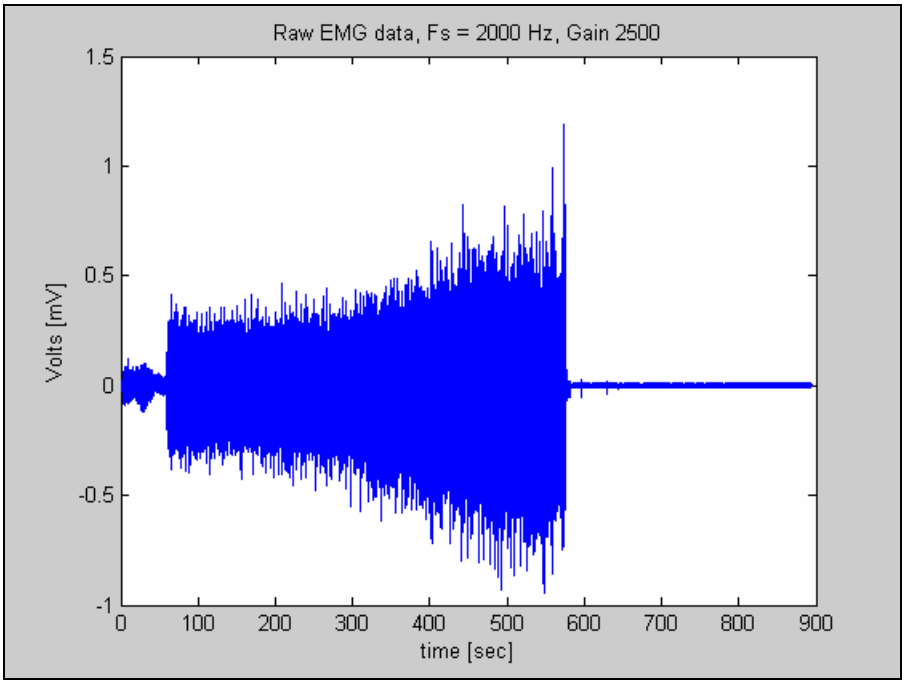

Figure B.1 Subject AK's Raw EMG measurement.

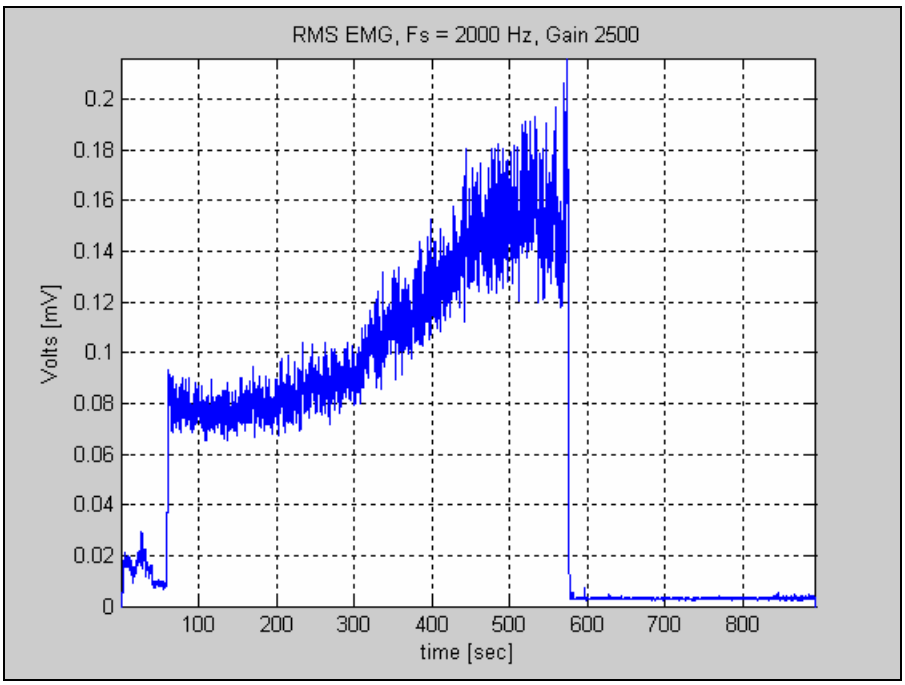

Figure B.2 Subject AK's RMS measurement.

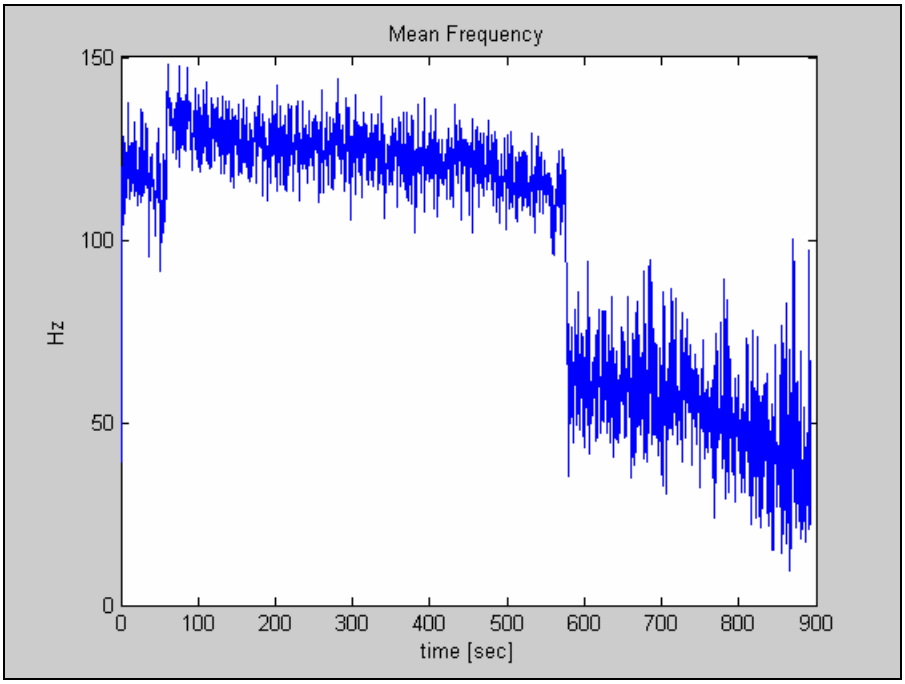

Figure B.3 Subject AK's MNF measurement.

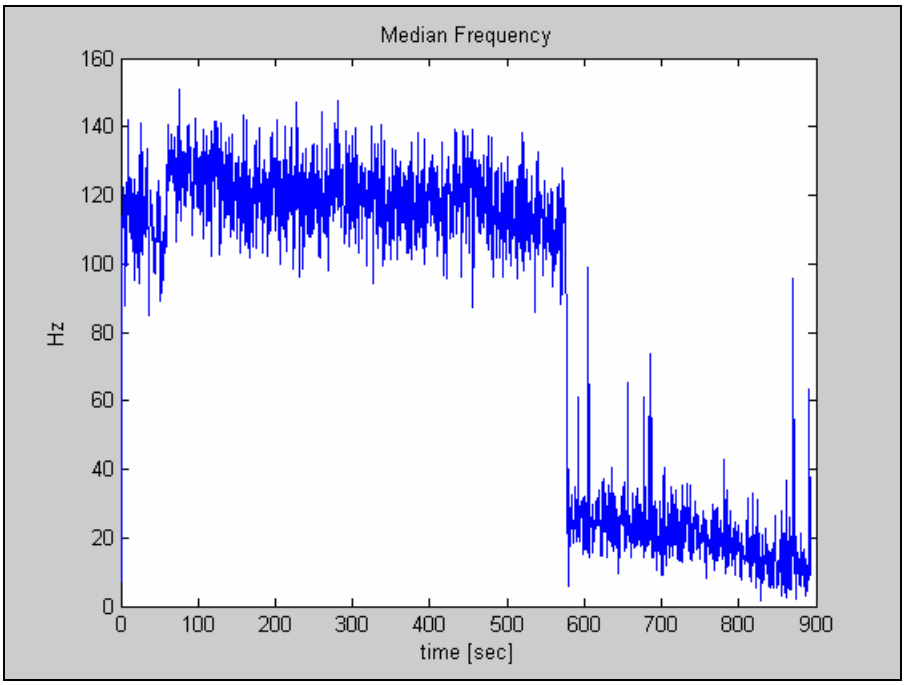

Figure B.4 Subject AK's MDF measurement.

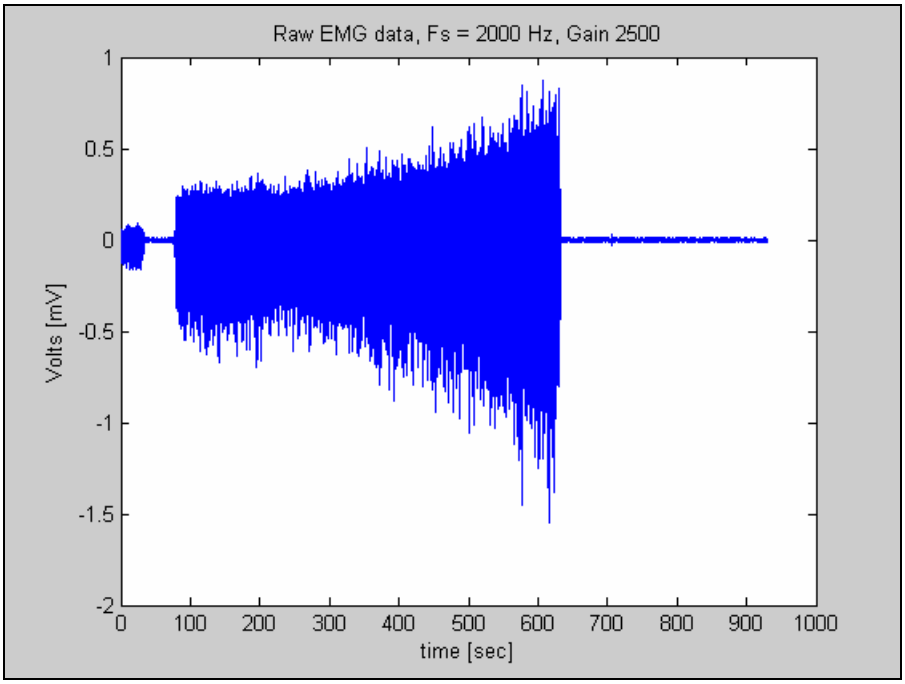

Figure B.5 Subject KS's Raw EMG measurement.

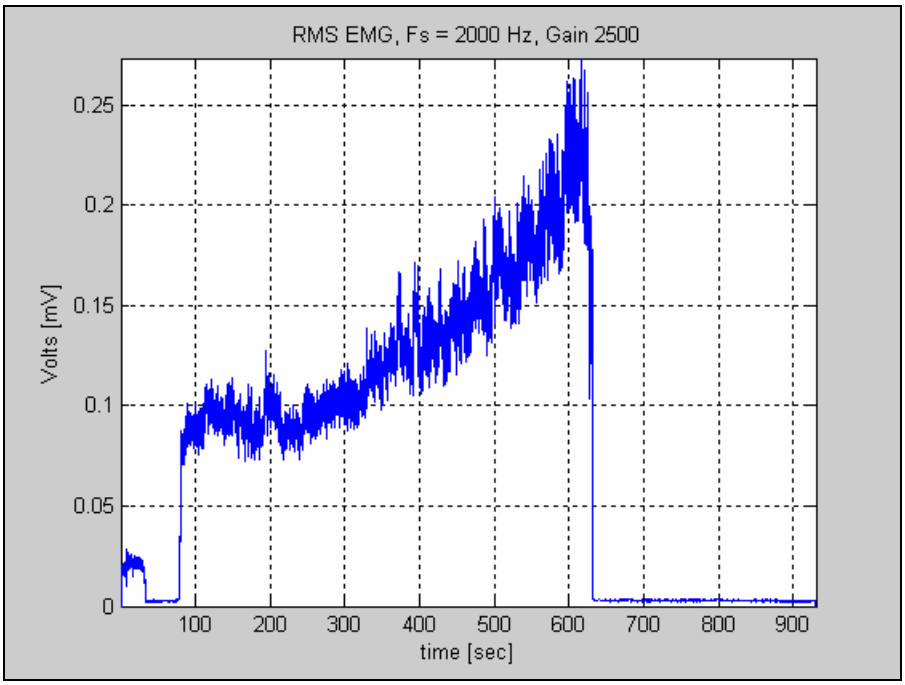

Figure B.6 Subject KS's RMS measurement.

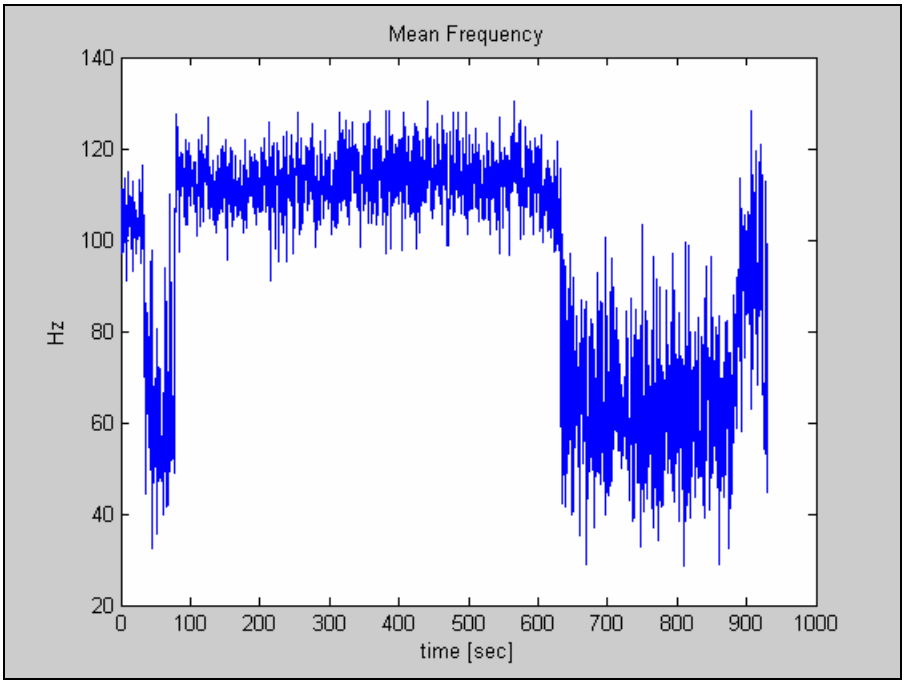

Figure B.7 Subject KS's MNF measurement.

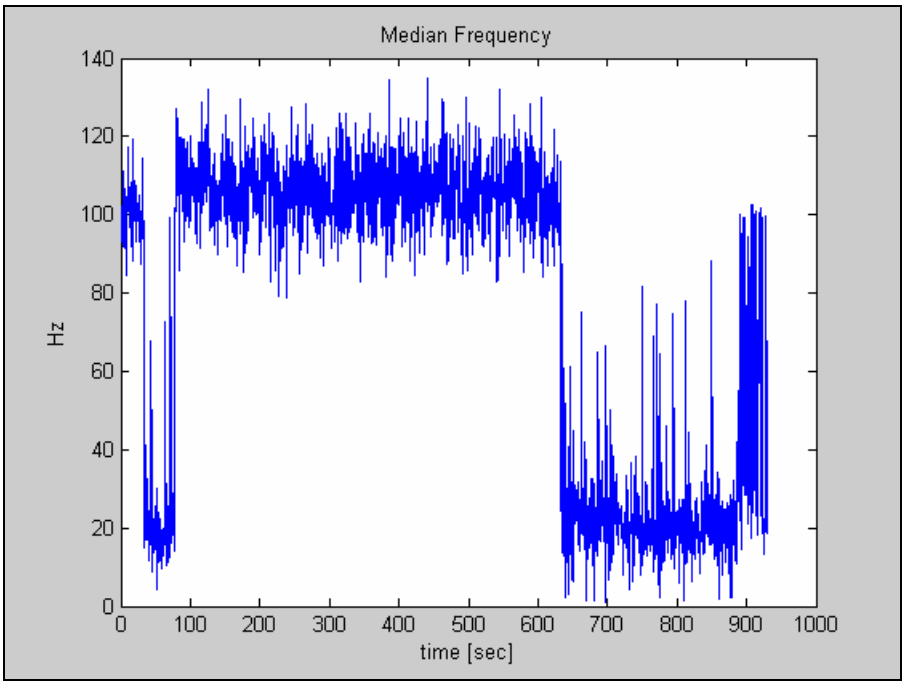

Figure B.8 Subject KS's MDF measurement.

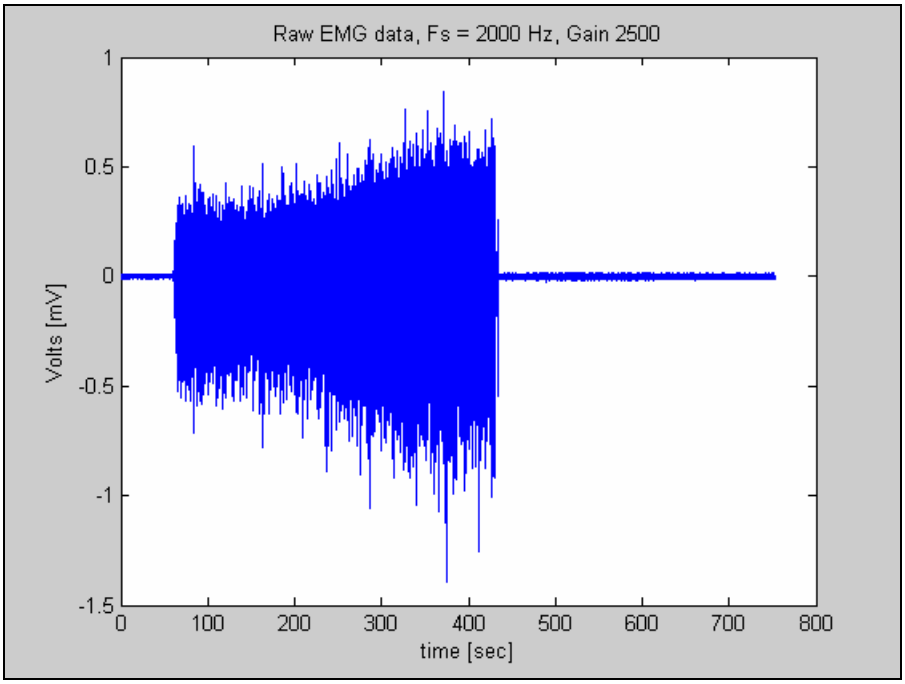

Figure B.9 Subject ME's Raw EMG measurement.

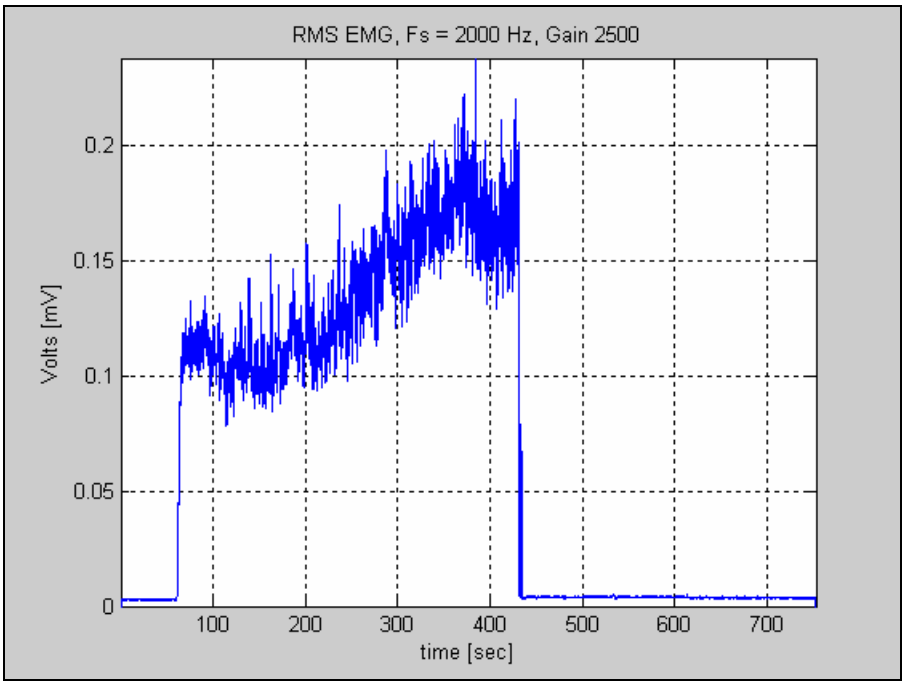

Figure B.10 Subject ME's RMS measurement.

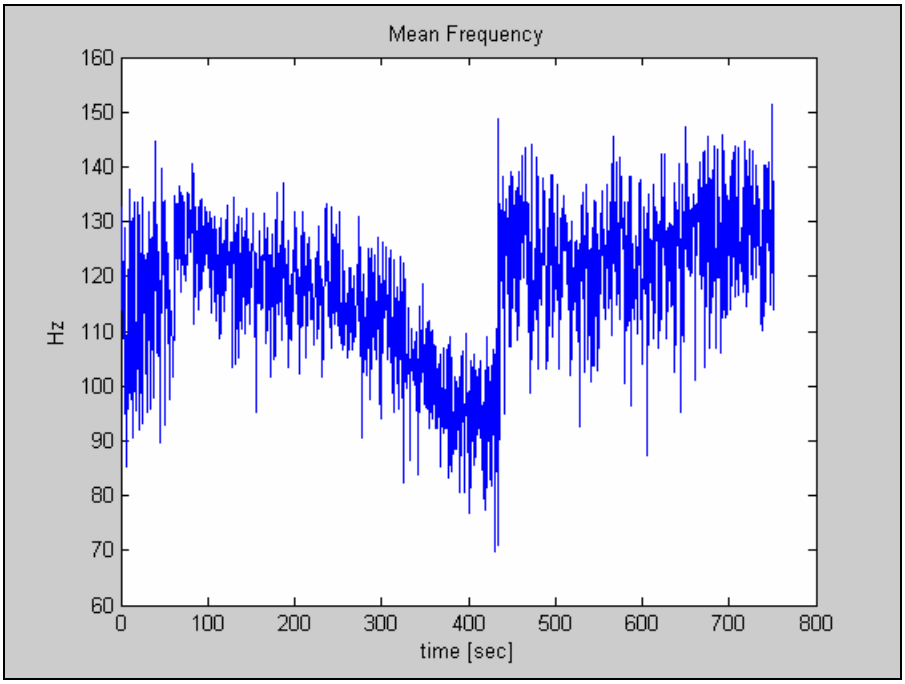

Figure B.11 Subject ME's MNF measurement.

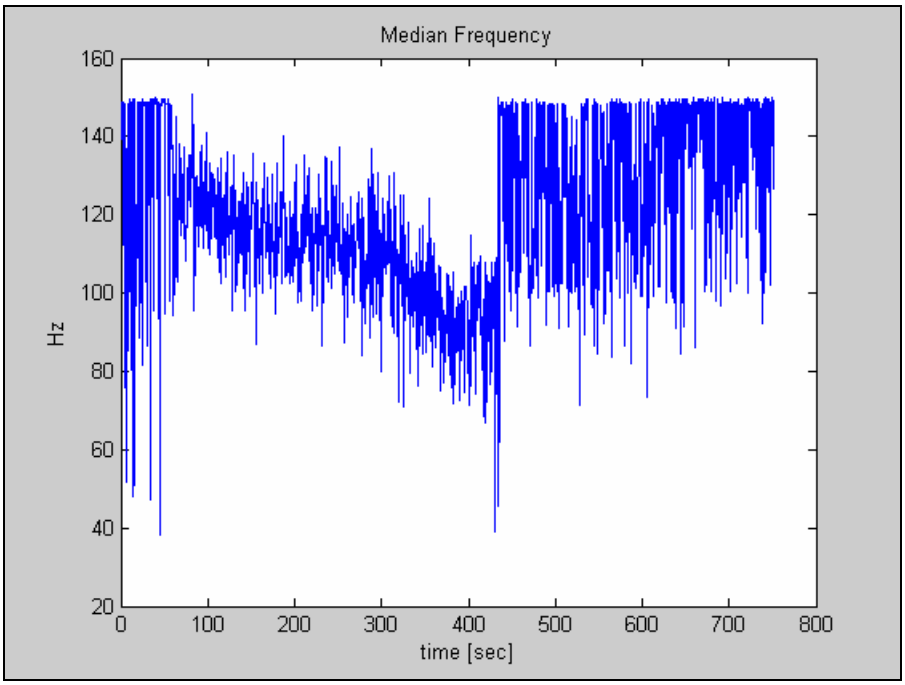

Figure B.12 Subject ME's MDF measurement.

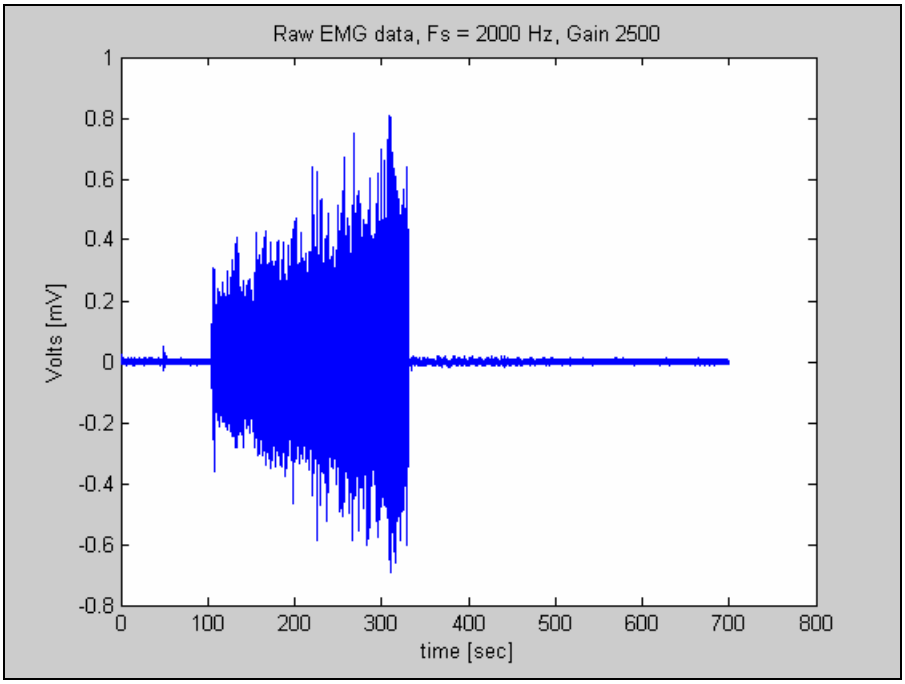

Figure B.13 Subject MH's Raw EMG measurement.

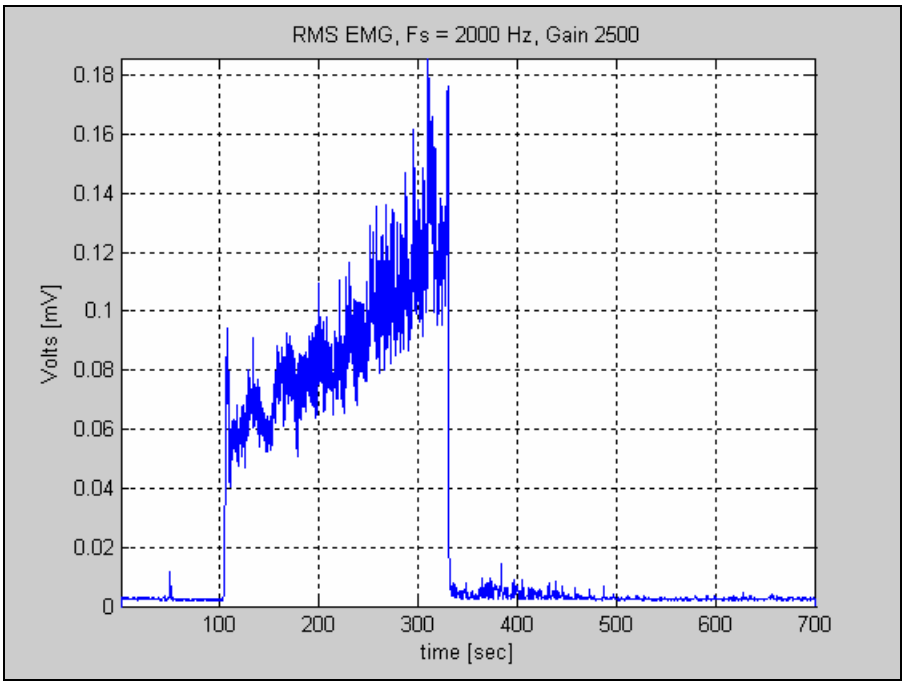

Figure B.14 Subject MH's RMS measurement.

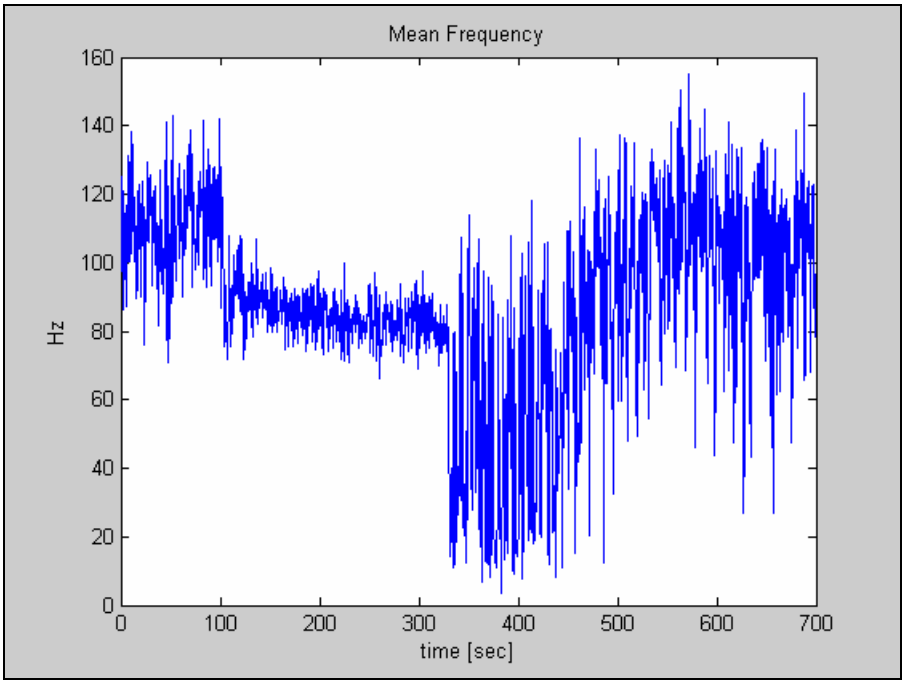

Figure B.15 Subject MH's MNF measurement.

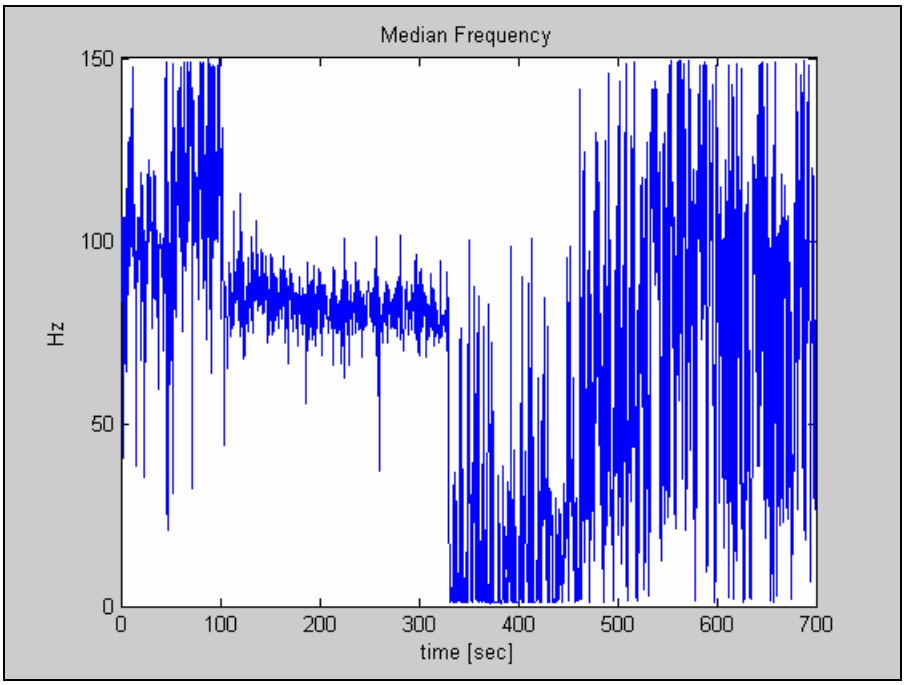

Figure B.16 Subject MH's MDF measurement.

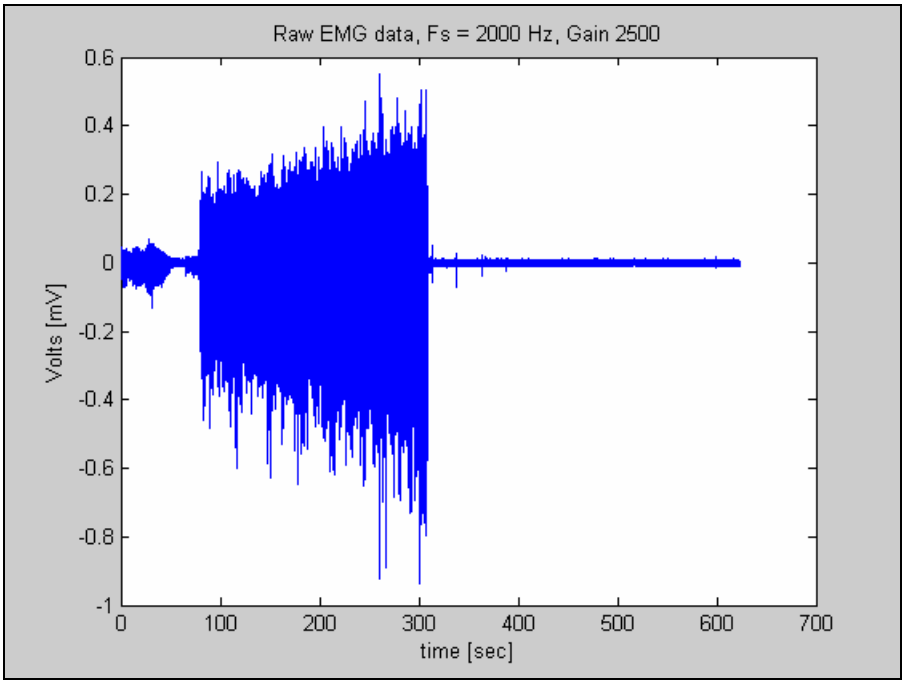

Figure B.17 Subject MP's Raw EMG measurement.

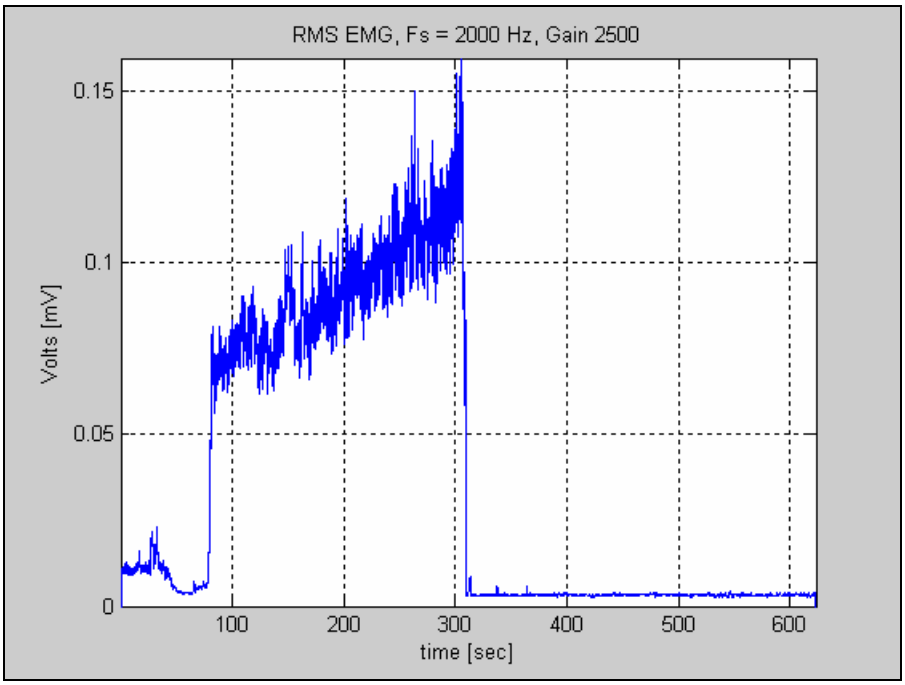

Figure B.18 Subject MP's RMS measurement.

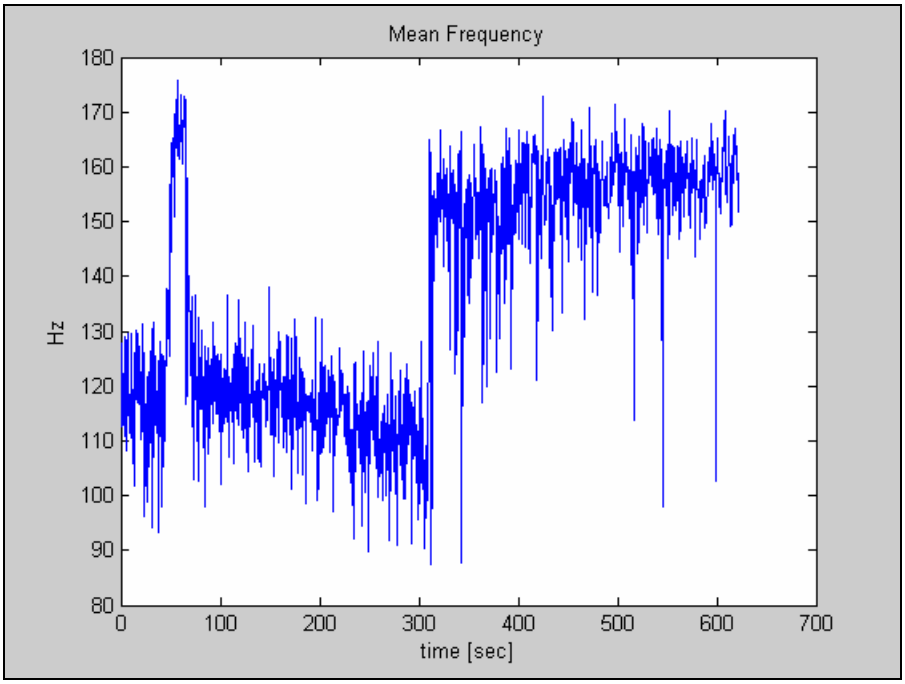

Figure B.19 Subject MP's MNF measurement.

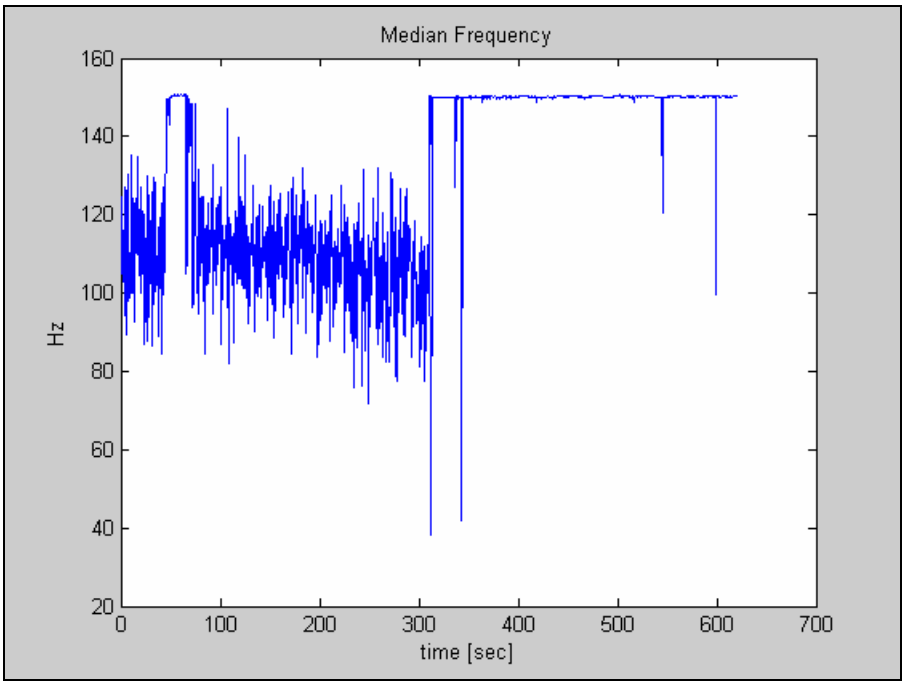

Figure B.20 Subject MP's MNF measurement.

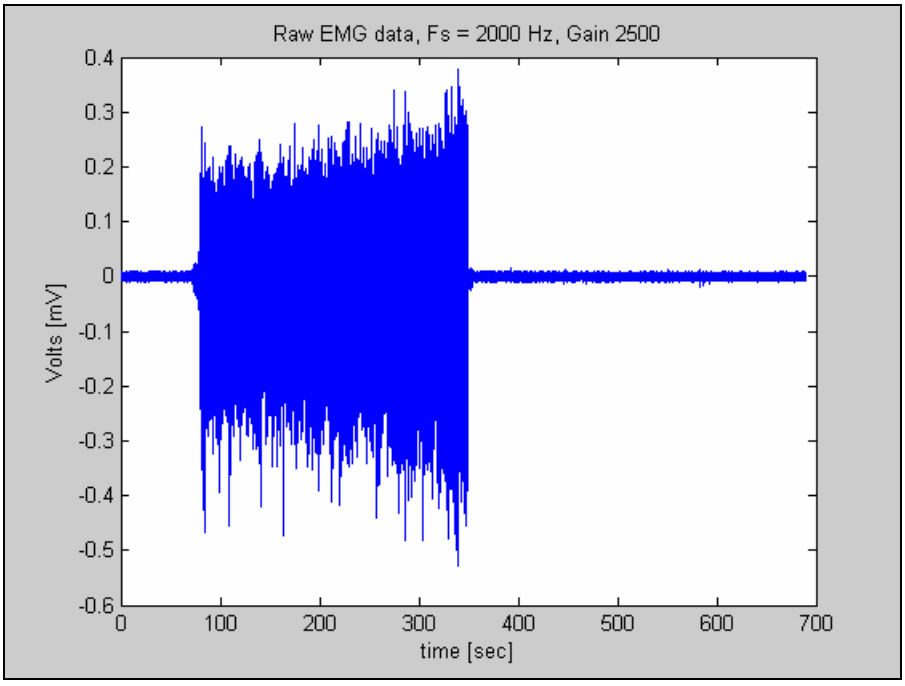

Figure B.21 Subject LT's Raw EMG measurement.

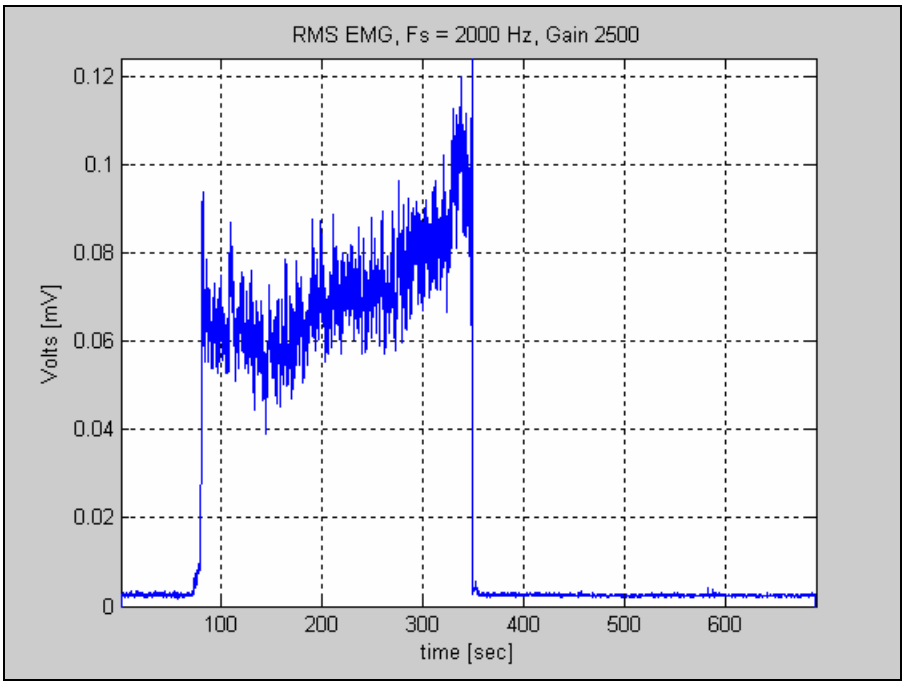

Figure B.22 Subject LT's RMS measurement.

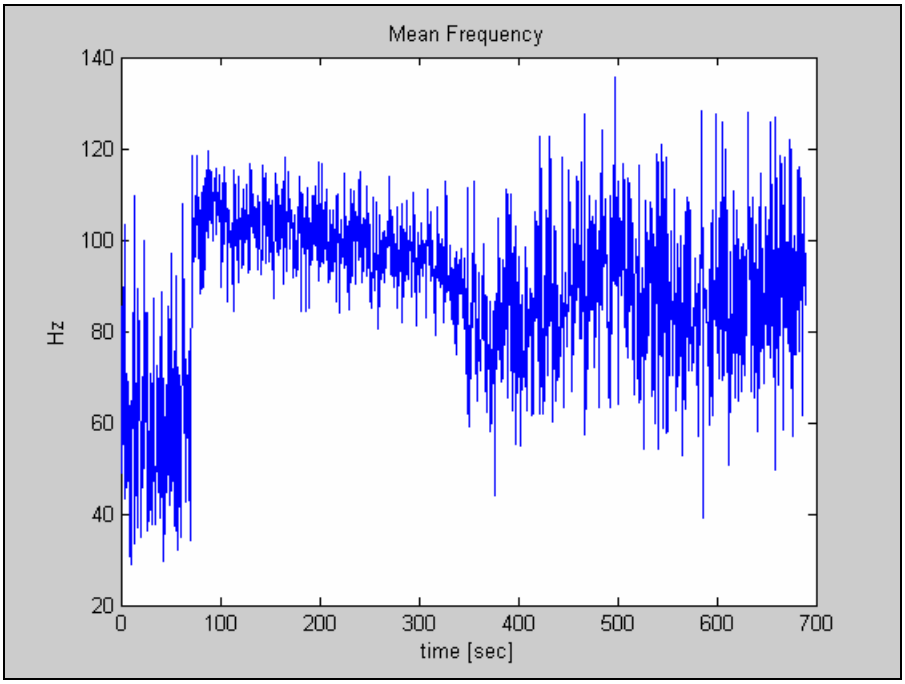

Figure B.23 Subject LT's MNF measurement.

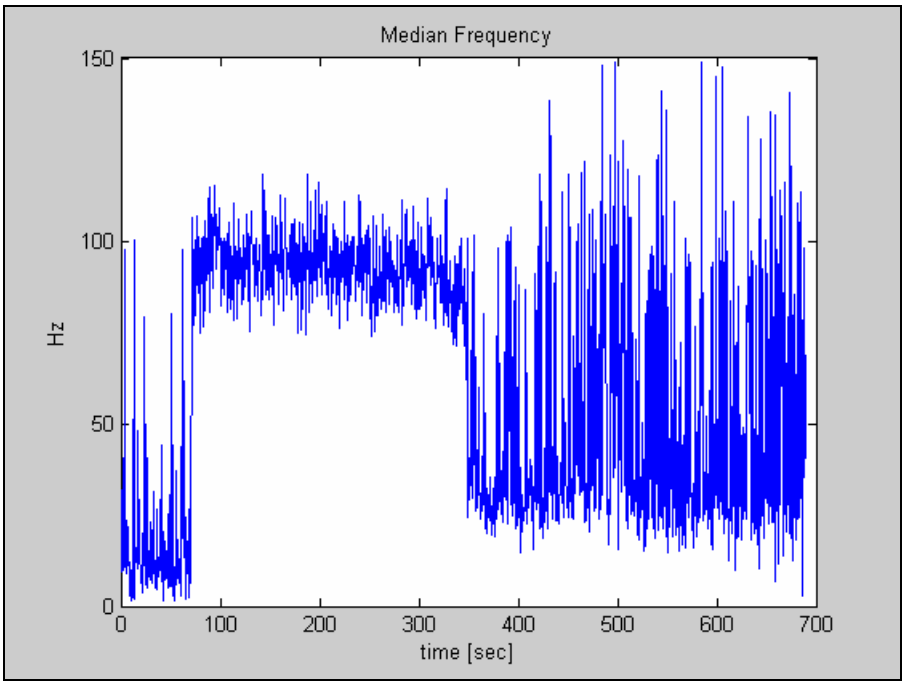

Figure B.24 Subject LT's MDF measurement.

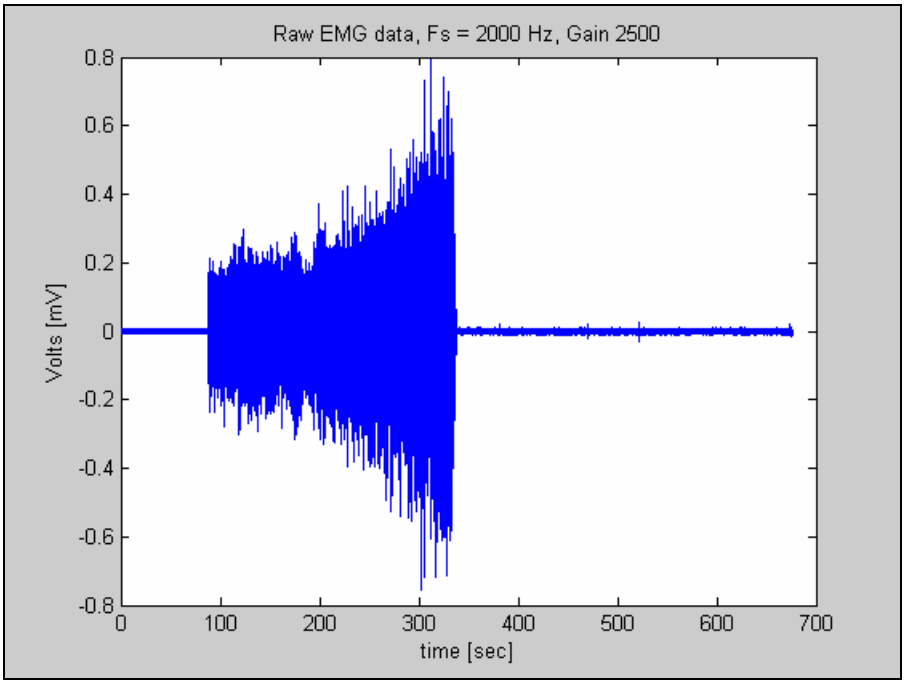

Figure B.25 Subject MA's Raw EMG measurement.

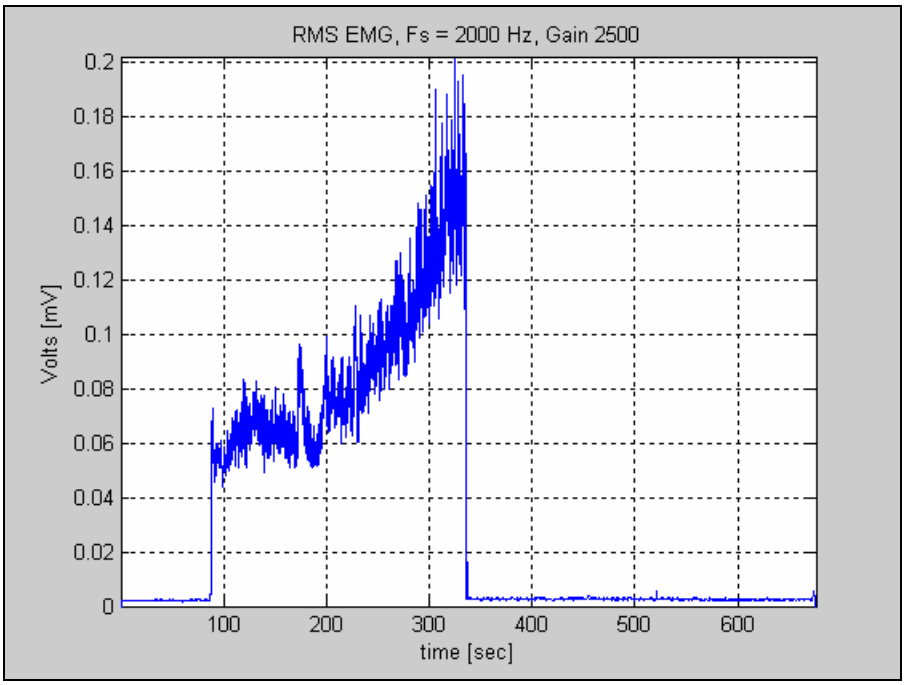

Figure B.26 Subject MA's RMS measurement.

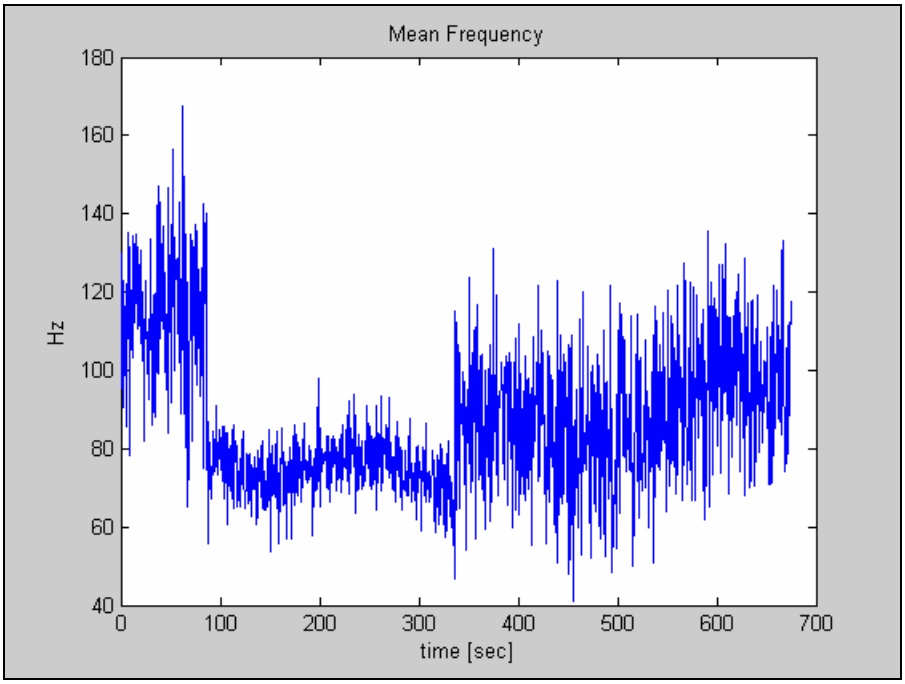

Figure B.27 Subject MA's MNF measurement.

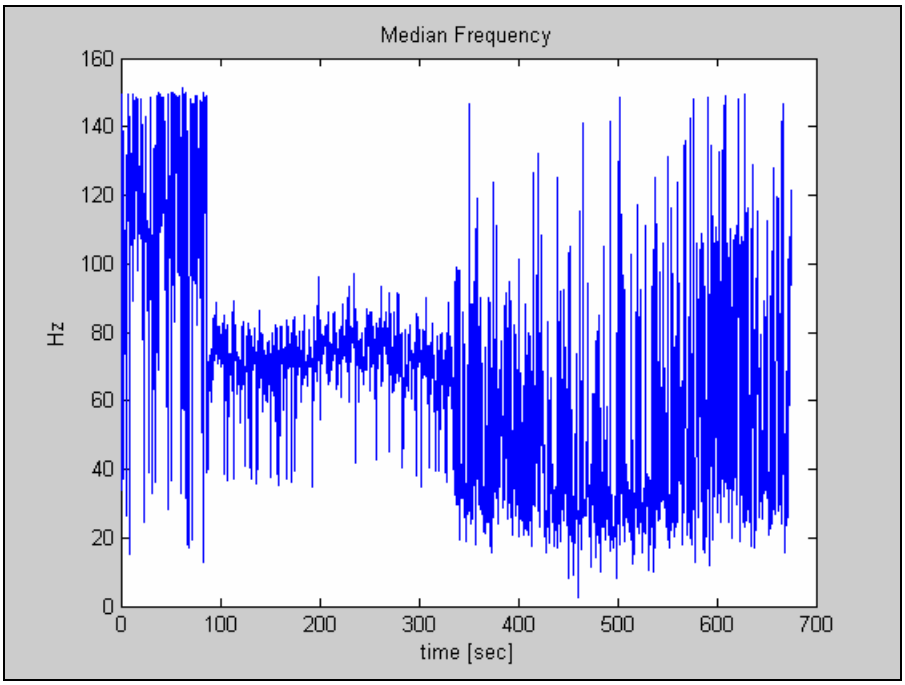

Figure B.28 Subject MA's MDF measurement.

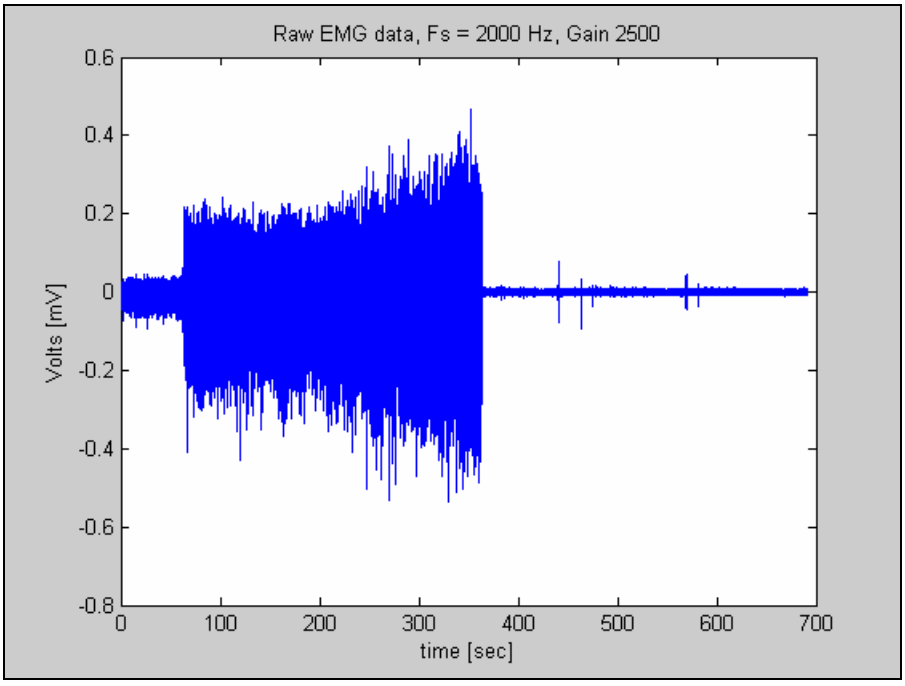

Figure B.29 Subject OC's Raw EMG measurement.

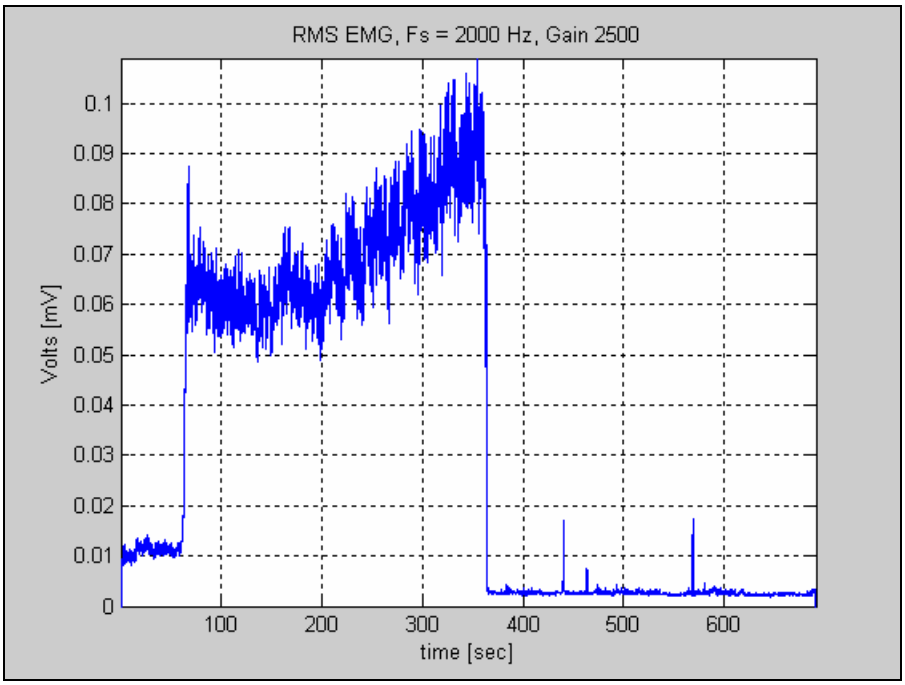

Figure B.30 Subject OC's RMS measurement.

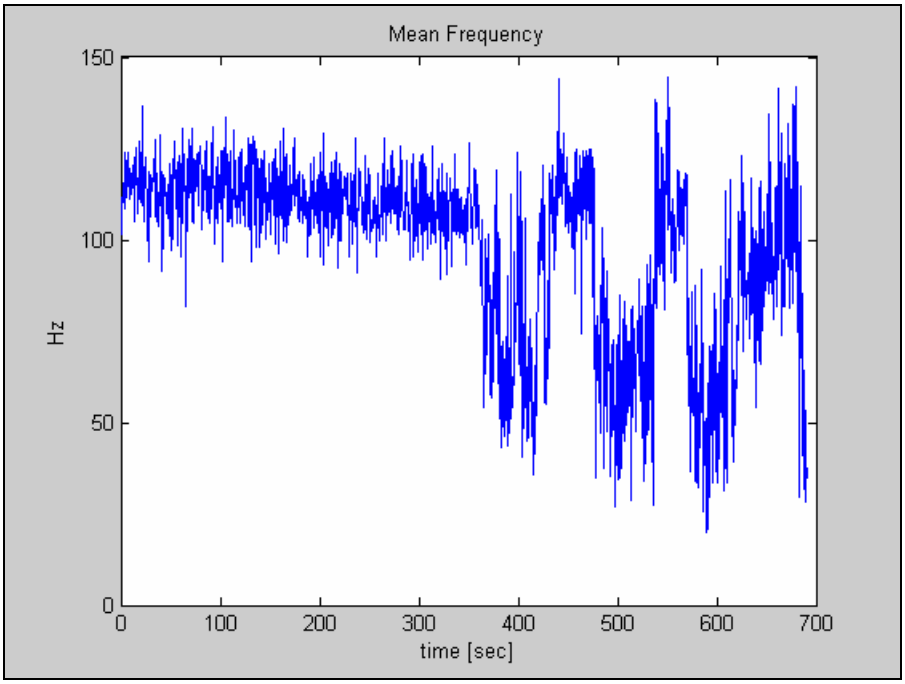

Figure B.31 Subject OC's MNF measurement.

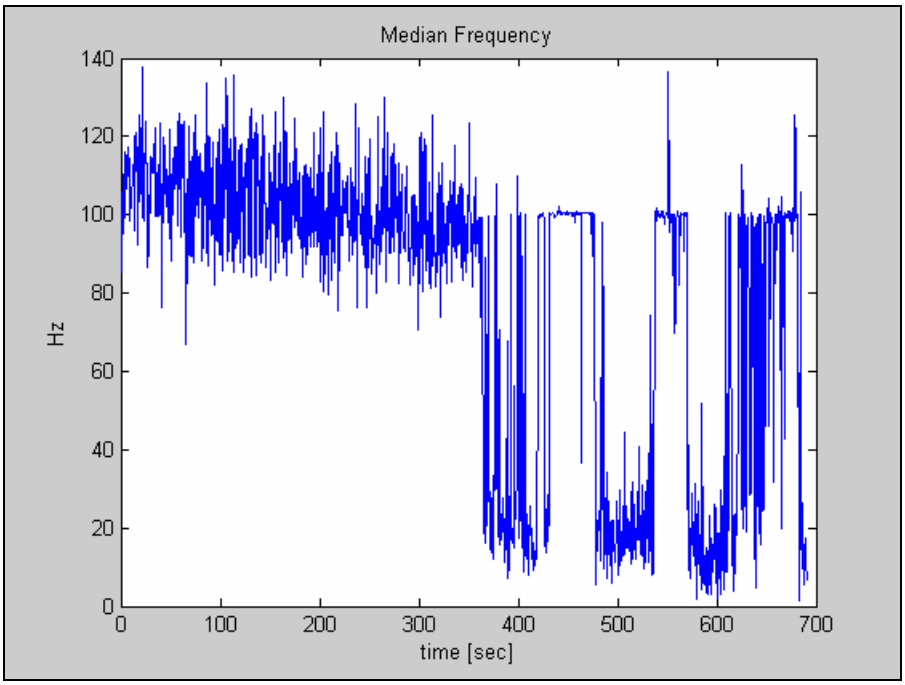

Figure B.32 Subject OC's MNF measurement.

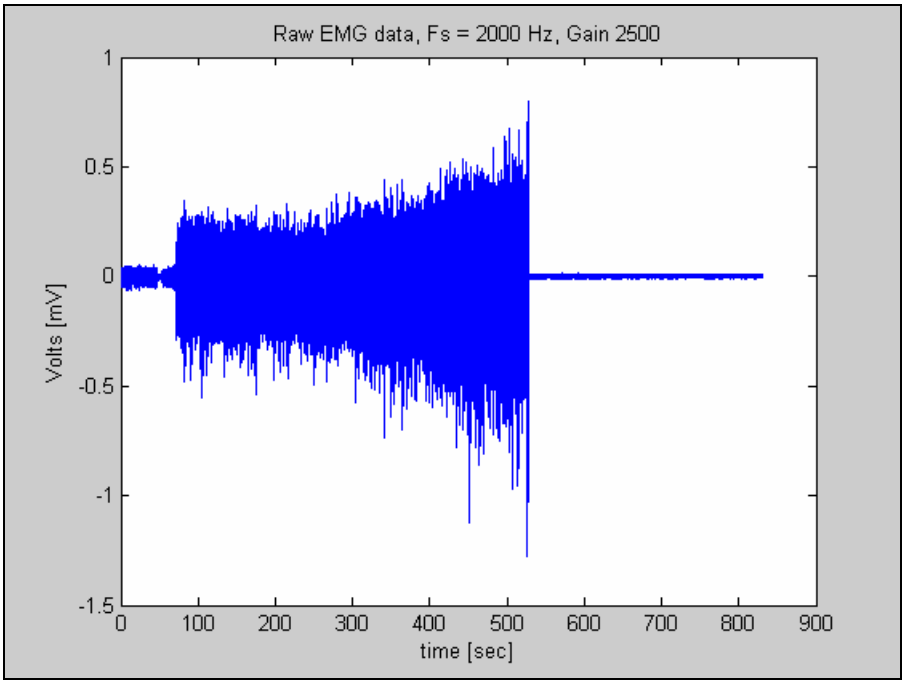

Figure B.33 Subject SaK's Raw EMG measurement.

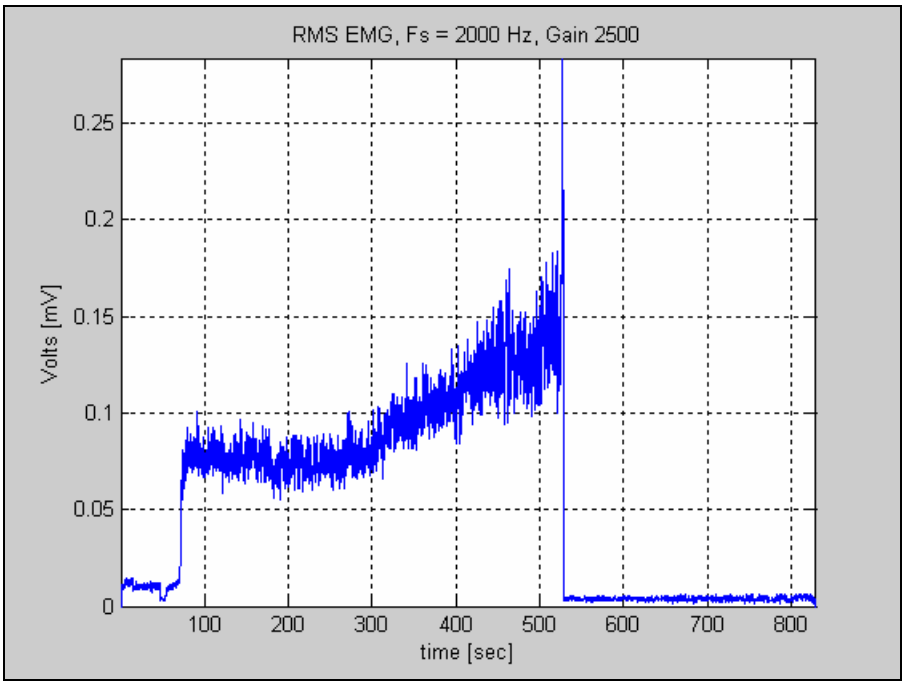

Figure B.34 Subject SaK's RMS measurement.

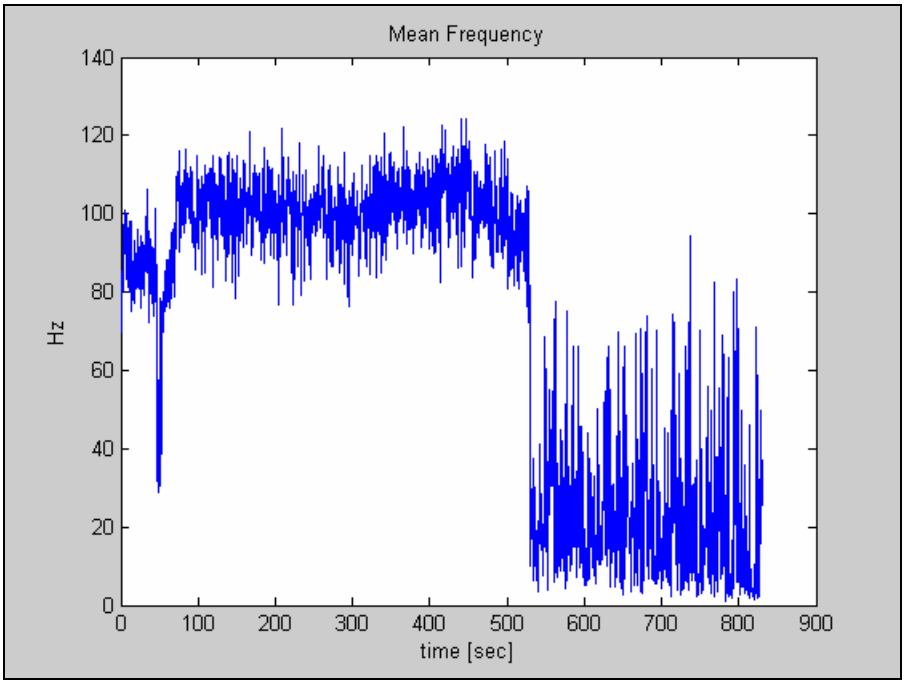

Figure B.35 Subject SaK's MNF measurement.

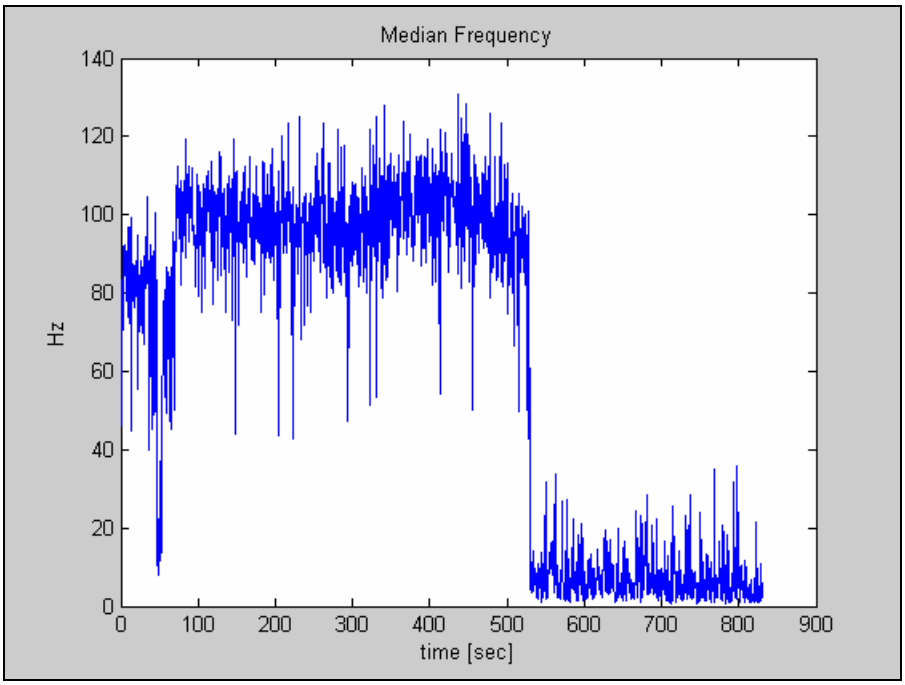

Figure B.36 Subject SaK's MDF measurement.

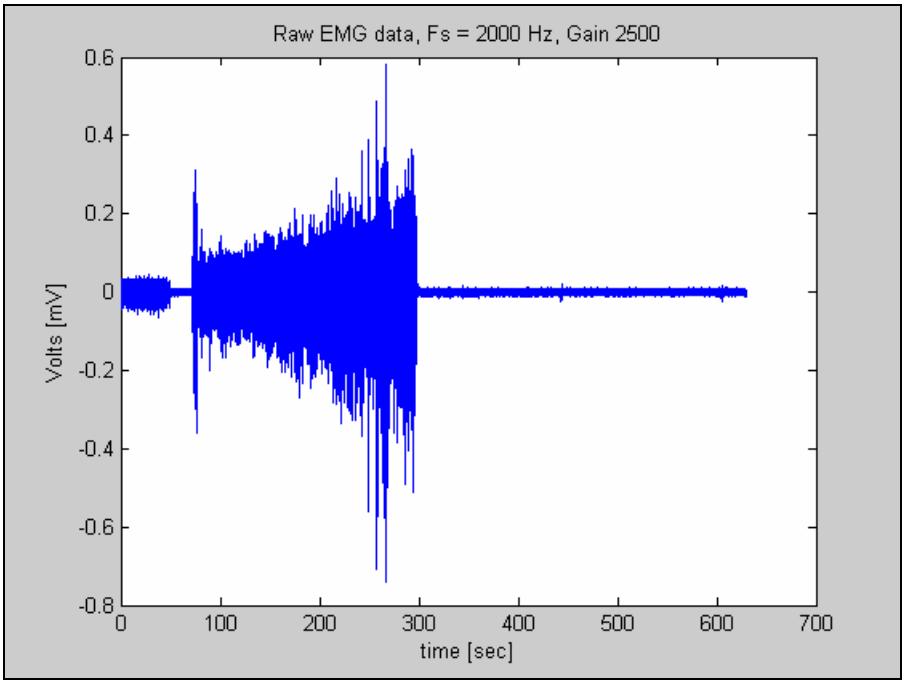

Figure B.37 Subject SeK's Raw EMG measurement.

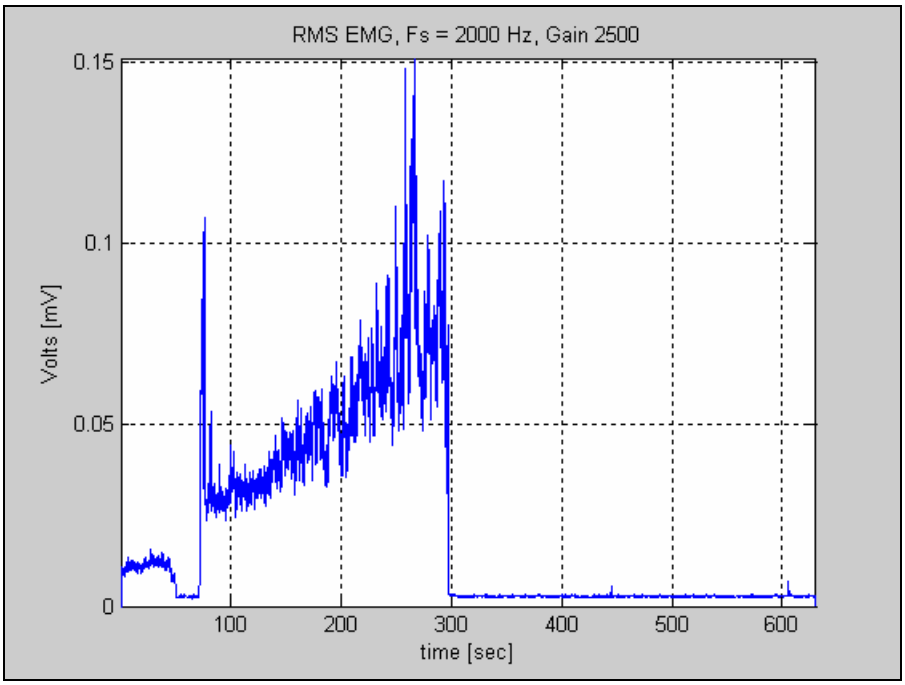

Figure B.38 Subject SeK's RMS measurement.

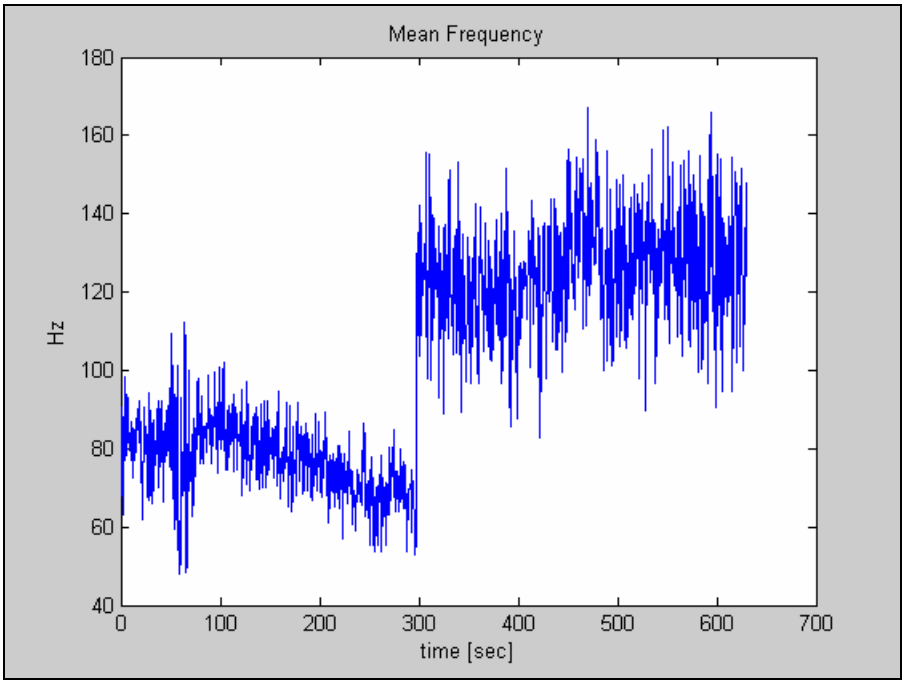

Figure B.39 Subject SeK's MNF measurement.

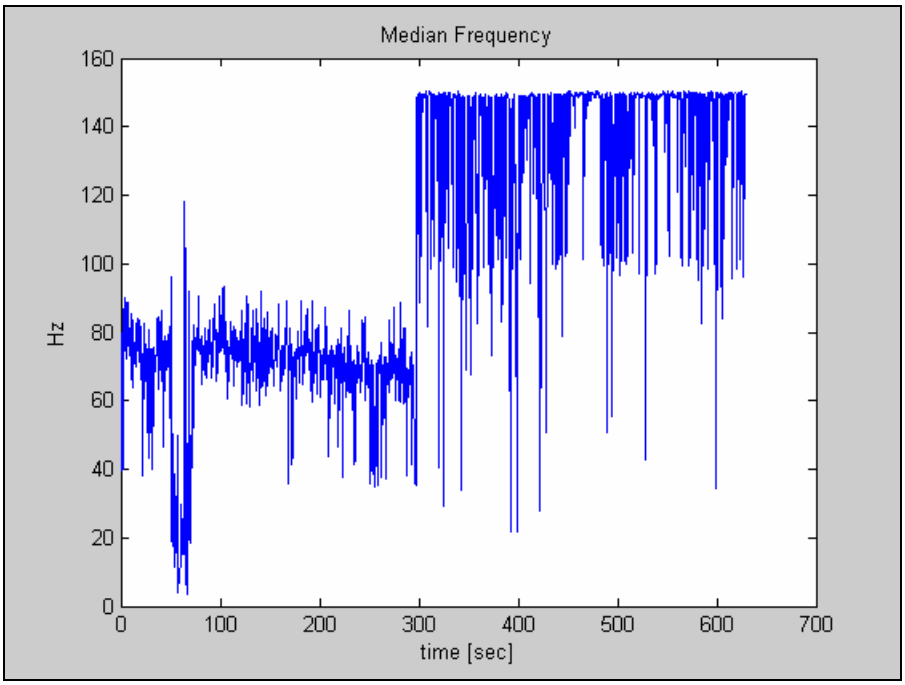

Figure B.40 Subject SeK's MDF measurement.

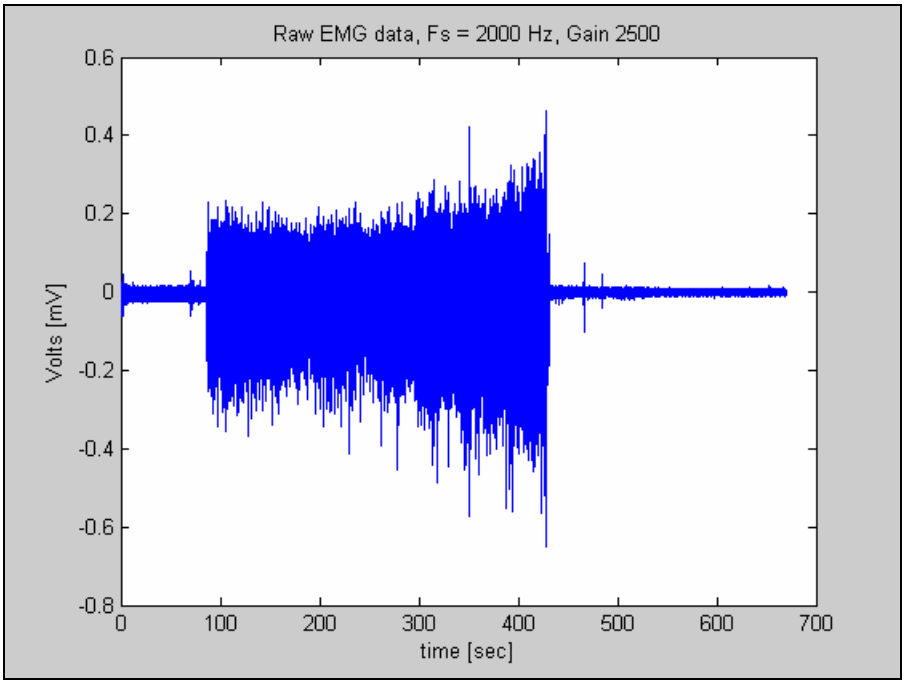

Figure B.41 Subject UA's Raw EMG measurement.

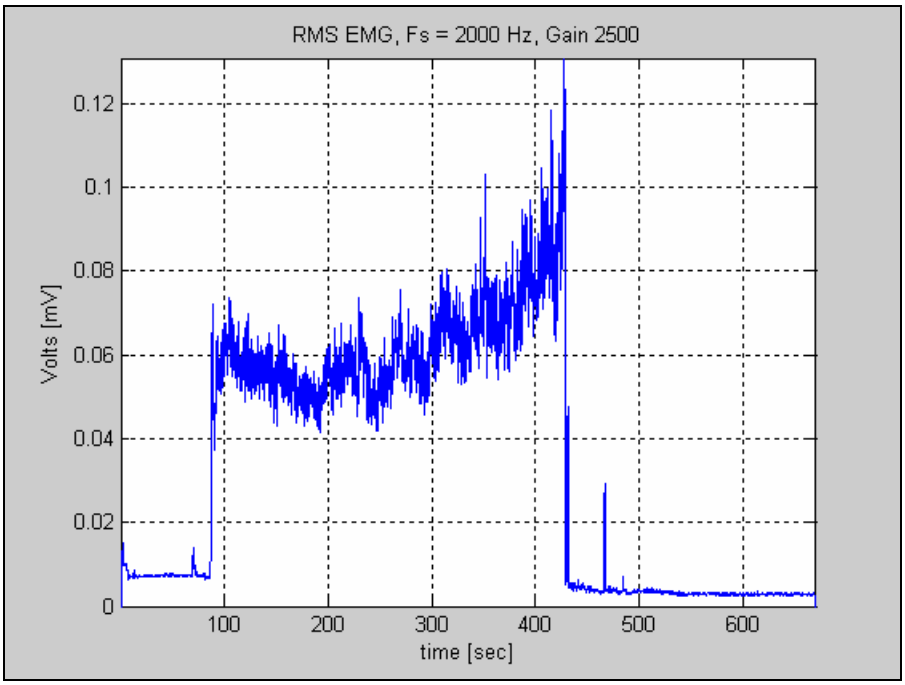

Figure B.42 Subject UA's RMS measurement.

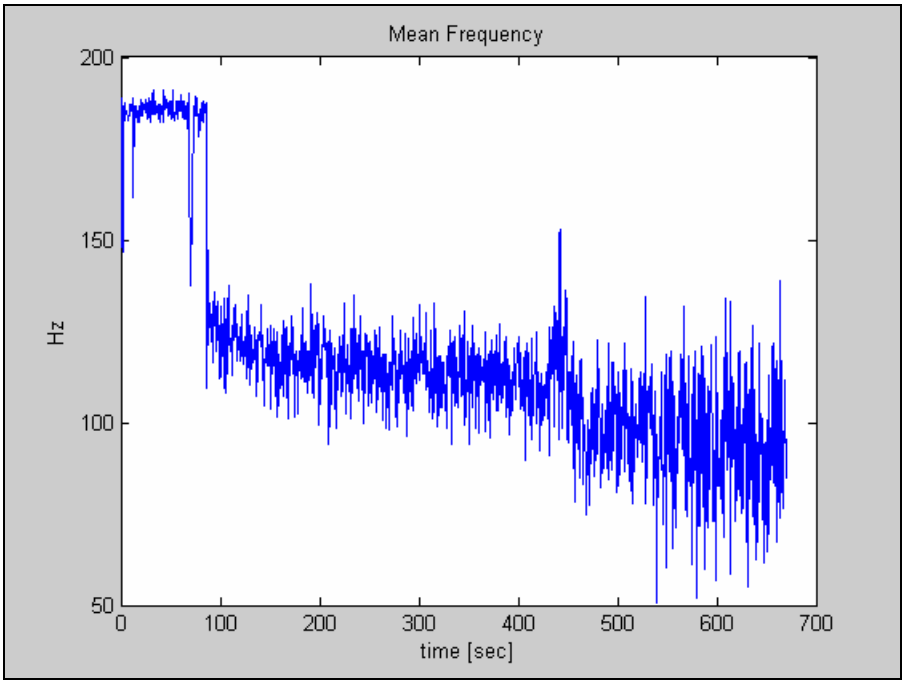

Figure B.43 Subject UA's MNF measurement.

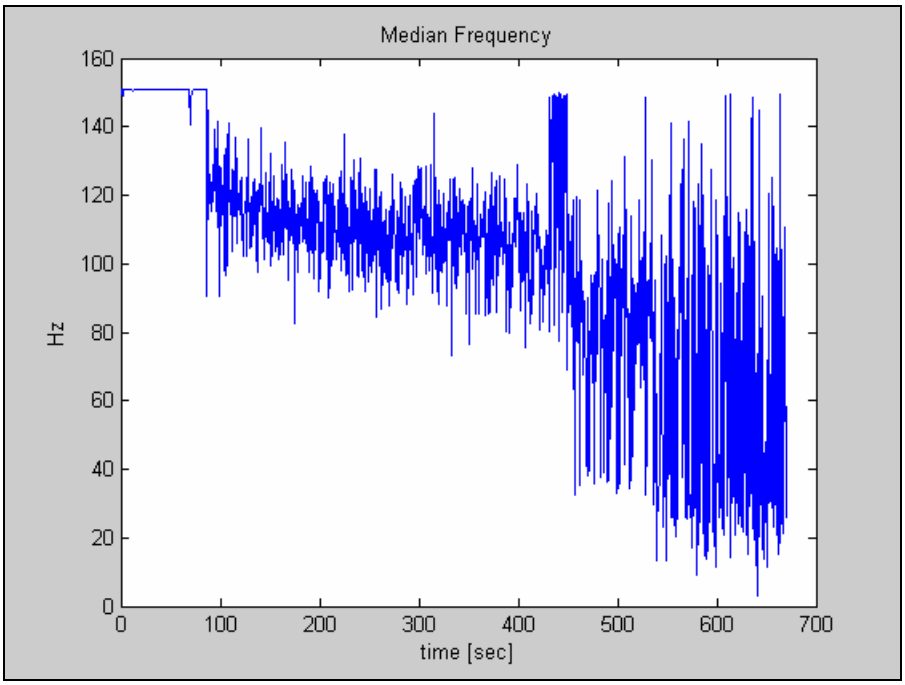

Figure B.44 Subject UA's MNF measurement.

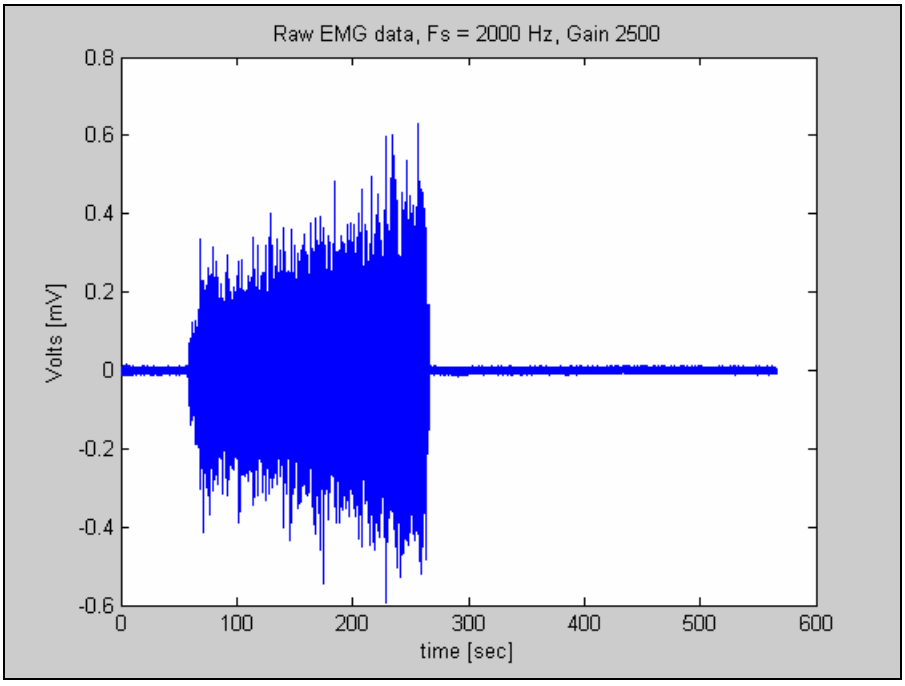

Figure B.45 Subject UE's Raw EMG measurement.

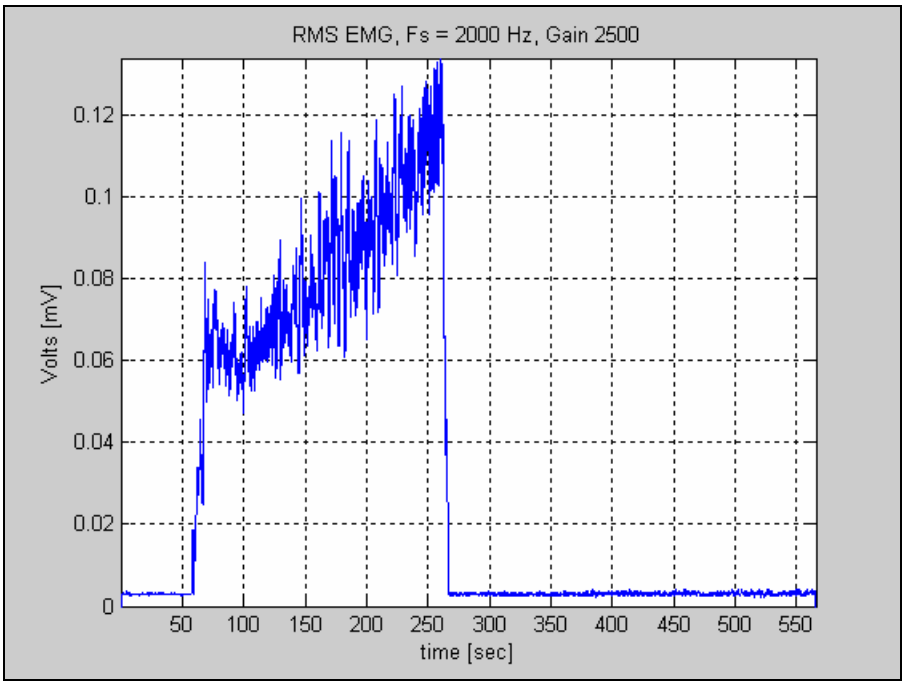

Figure B.46 Subject UE's RMS measurement.

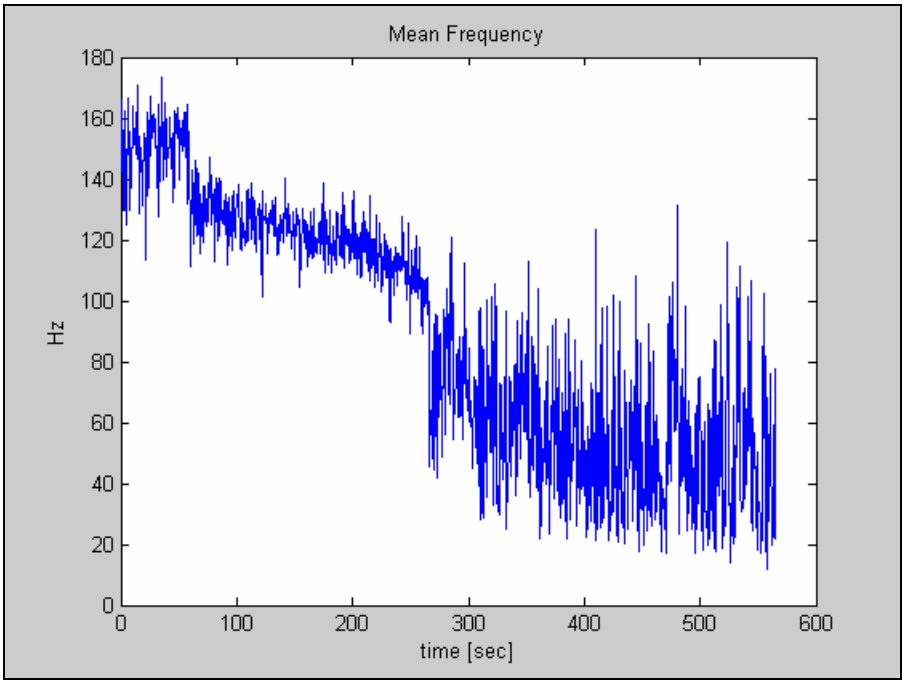

Figure B.47 Subject UE's MNF measurement.

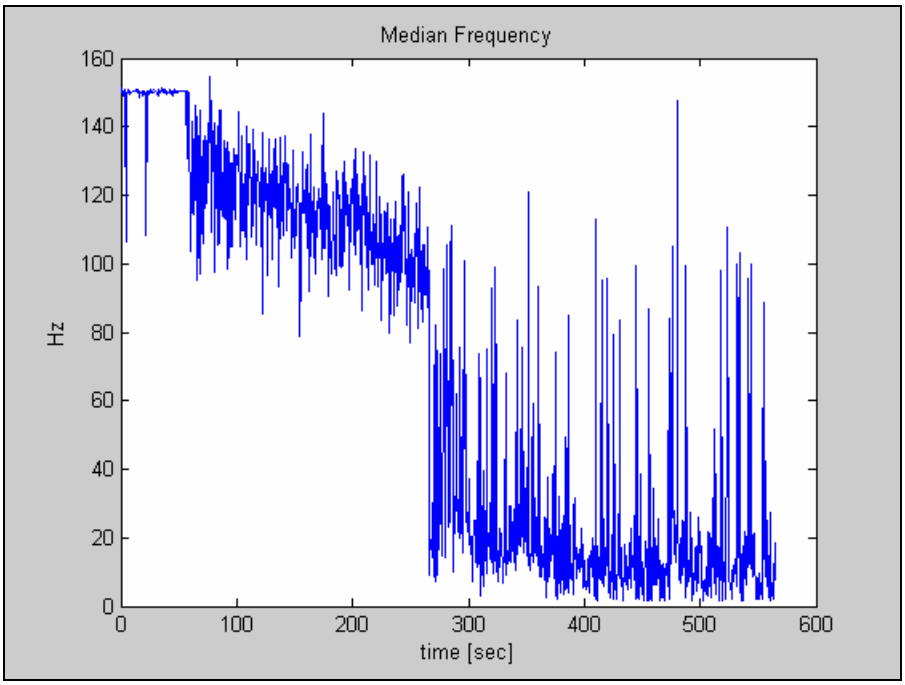

Figure B.48 Subject UE's MDF measurement.

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